

## Macrophages in the Rete Testis of Pubertal Male Fowl

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Macrophages are known to be present in various segments of the testicular excurrent duct system of mammals [3-5]. In the excurrent duct system of birds, the presence of macrophages has been reported in the rete testes of fowl and drake, as well [1, 2, 6]. In the previous reports, macrophages were examined in sexually matured animals, but the presence of macrophages at puberty has not been examined. The present histological study was carried out to clarify when macrophages would appear in the rete testis using pubertal male fowls.

Twenty-three male White Leghorn fowls, 14 to 16 weeks of age, were used in this study. For light microscopy, the testes and epididymides were excised and fixed in 10% formalin. Four  $\mu\text{m}$  paraffin sections were prepared by routine methods and were stained with hematoxylin and eosin. For transmission electron microscopy, small tissue blocks of the epididymides were fixed in cacodylate-buffered 2.5% glutaraldehyde for at least 2 hr, postfixed in cacodylate-buffered 1%  $\text{OsO}_4$  for 2 hr, and then embedded in Quetol 812 (Nisshin EM Co., Ltd., Tokyo). Ultrathin sections were stained doubly with uranyl acetate and lead citrate. They were examined by a Hitachi H-800MU transmission electron microscope.

In 2 of the 23 fowls examined, the seminiferous epithelium was composed mainly of supporting cells and spermatogonia. The lumen of the rete testis was empty except for a few free cells of unknown nature.

In 9 of the 23 fowls, cell divisions of spermatogenic cells were observed at various frequencies within the seminiferous tubules. Spermatids were found in some cases, but no spermatozoa were seen in the rete testis. Free cells, probably desquamated immature spermatogenic cells, were observed in the lumen of the rete testis. These desquamated cells were more numerous in the fowls showing more advanced stages of spermatogenesis. Another type of free cells differing from the desquamated cells was found in the rete testis. These cells were oval, elongated or irregular in shape, often provided with eccentric nuclei. The cytoplasm contained one or more large vacuoles in which the desquamated cells were occasionally observed (Fig. 1a). Transmission electron microscopy of the free cells revealed numerous cytoplasmic processes at the cell margin and lysosomal dense bodies and vacuoles in the cytoplasm; degenerated cells were seen in the vacuoles (Fig. 1b). These nonspermatogenic cells were identified as macrophages.

In the remaining 12 fowls, spermatozoa were found in various numbers within the seminiferous tubules. Desquamated immature spermatogenic cells, spermatozoa, cell debris and macrophages in the rete testis were more numerous than in the above-mentioned cases. Some macrophages were suspended in the lumen and others were on the epithelium; both often contained the desquamated cells and spermatozoa (Fig. 2a). Masses of macrophages with foamy cytoplasm were seen in the lumen of the rete testis. Ultrastructurally, macrophages

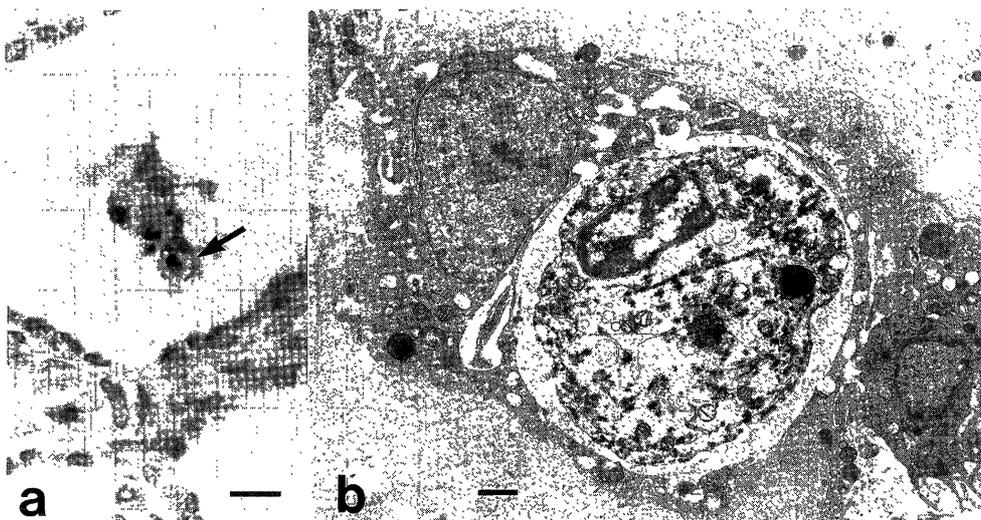


Fig. 1. Light (a) and electron (b) micrographs of macrophages within the lumen of the rete testis containing few spermatozoa. a: A macrophage (arrow) contains a nucleus within a vacuole. Bar=10  $\mu\text{m}$ . b: A degenerated cell is seen in the vacuole of a macrophage. Bar=1  $\mu\text{m}$ .

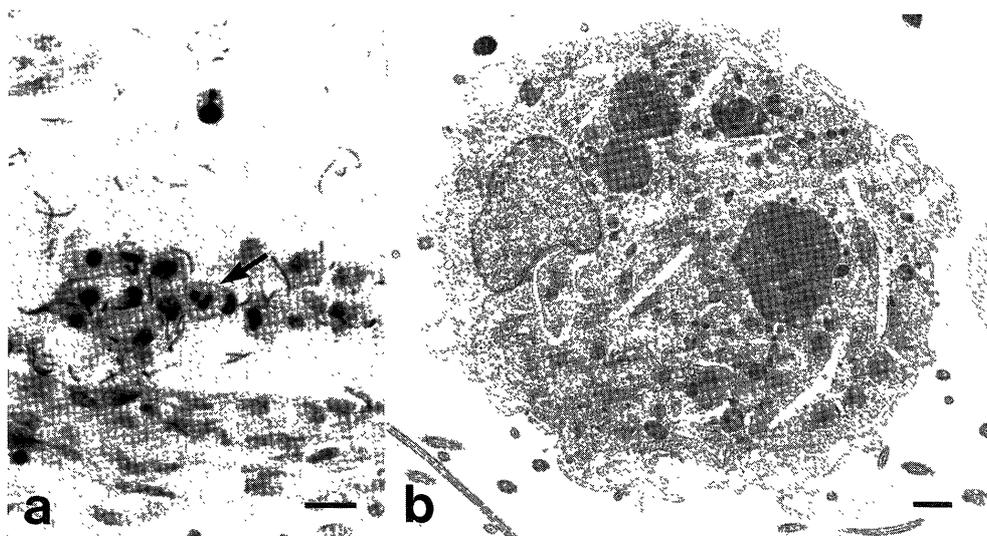


Fig. 2. Light (a) and electron (b) micrographs of macrophages within the lumen of the rete testis containing a lot of spermatozoa.

a: A macrophage (arrow) contains three nuclei, probably those of desquamated immature spermatogenic cells, and the others contain spermatozoa. Bar=10  $\mu$ m.

b: A macrophage contains well-developed lysosomal dense bodies of various sizes in the cytoplasm. Bar=1  $\mu$ m.

were irregular in outline and had slender microvilli of various lengths and shapes at the cell margin (Fig. 2b). Lysosomal dense bodies, vacuoles, multivesicular bodies and fragments of spermatozoa were observed in the cytoplasm.

The present results show that macrophages appear in the rete testis when seminal fluid including desquamated immature spermatogenic cells and spermatozoa flows into the excurrent duct system. The reason for this correspondence is unclear. The development of the epididymis depends on androgen [7], which may have some influence upon the movement of macrophages in this organ. On the other hand, it is possible that some components of seminal fluid may cause macrophages to migrate into the rete testis. What the components are and how they reach the macrophages remain to be clarified.

Roussel *et al.* [8] considered that macrophages might be involved in the removal of abnormal spermatozoa. In mature fowls, a large number of spermatids at different stages of maturation are present in the rete testis, a few are present in the efferent ductules and very few in the connecting ductules [9]. Spermatozoa and desquamated immature spermatogenic cells were phagocytized by mac-

rophages as shown in the present study. These findings suggest that macrophages are engaged in removal of not only spermatozoa but also immature spermatogenic cells in the seminal fluid.

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