Trans. Nat. Acad. Sci. & Tech. (Phils.)
1987.9:209-221

SPERMATOGENESIS IN *TILAPIA NILOTICA*: AN ULTRASTRUCTURE STUDY

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ABSTRACT

Spermatogenesis in *T. nilotica* was studied using an electron microscope. At the time of hatching primordial germ cells (PGC) with their characteristic germinal dense bodies are identified in the gut, their extra-gonadal origin. By 8-9 days posthatching, the PGCs have reached to the bipotential gonad primordia via the splanchnic mesoderm.

At days 16-20, onset of testis differentiation occurs by the appearance of stromal cavities between PGC clusters. Seminiferous tubules with spermatogonia are first recognized at day 40, and by day 53, primary spermatocytes first appear. By day 88, abundant fully differentiated sperm cells appear in the testis ducts. The ultrastructure of spermatogenesis in *Tilapia nilotica* is basically similar to that of other male vertebrates.

Introduction

Tilapia has become a prominent aquaculture organism on a global scale. In Israel and other countries where reproductive activity stops during winter followed by regression and development, electron microscope study has been done on testis recrudescence (Grier and Abraham, 1983). In tropical areas where reproductive activity is year long, ultrastructure research on testis and ovary differentiation in relation to pituitary histogenesis has been done (Herrera, 1986) in addition to light microscope study of testis structure (Hyder, 1970).

This study presents the ultrastructure of the first spermatogenesis during ontogeny in *Tilapia nilotica*. It provides baseline information that aids aquaculturists in problems on sex reversal, induced maturity and artificial breeding.

Materials and Methods

Tilapia eggs and embryos were secured from the Institute of Fisheries Development and Research, University of the Philippines. These were cultured in plastic basins filled with dechlorinated tap water to about 2/3 their volume and aerated continuously.

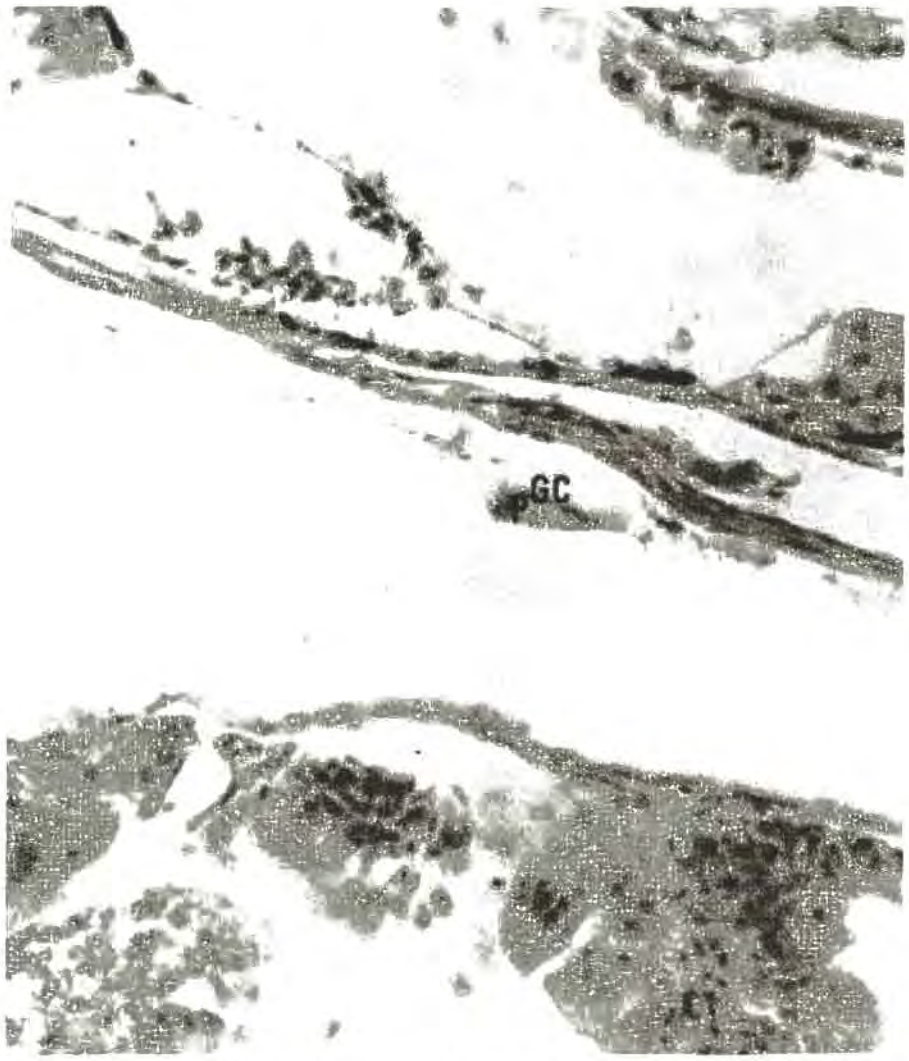


Fig. 1. The primordial germ cell in the dorsal mesentery on its way from the endoderm and splanchnic mesoderm. Hematoxylin-eosin. x 100.

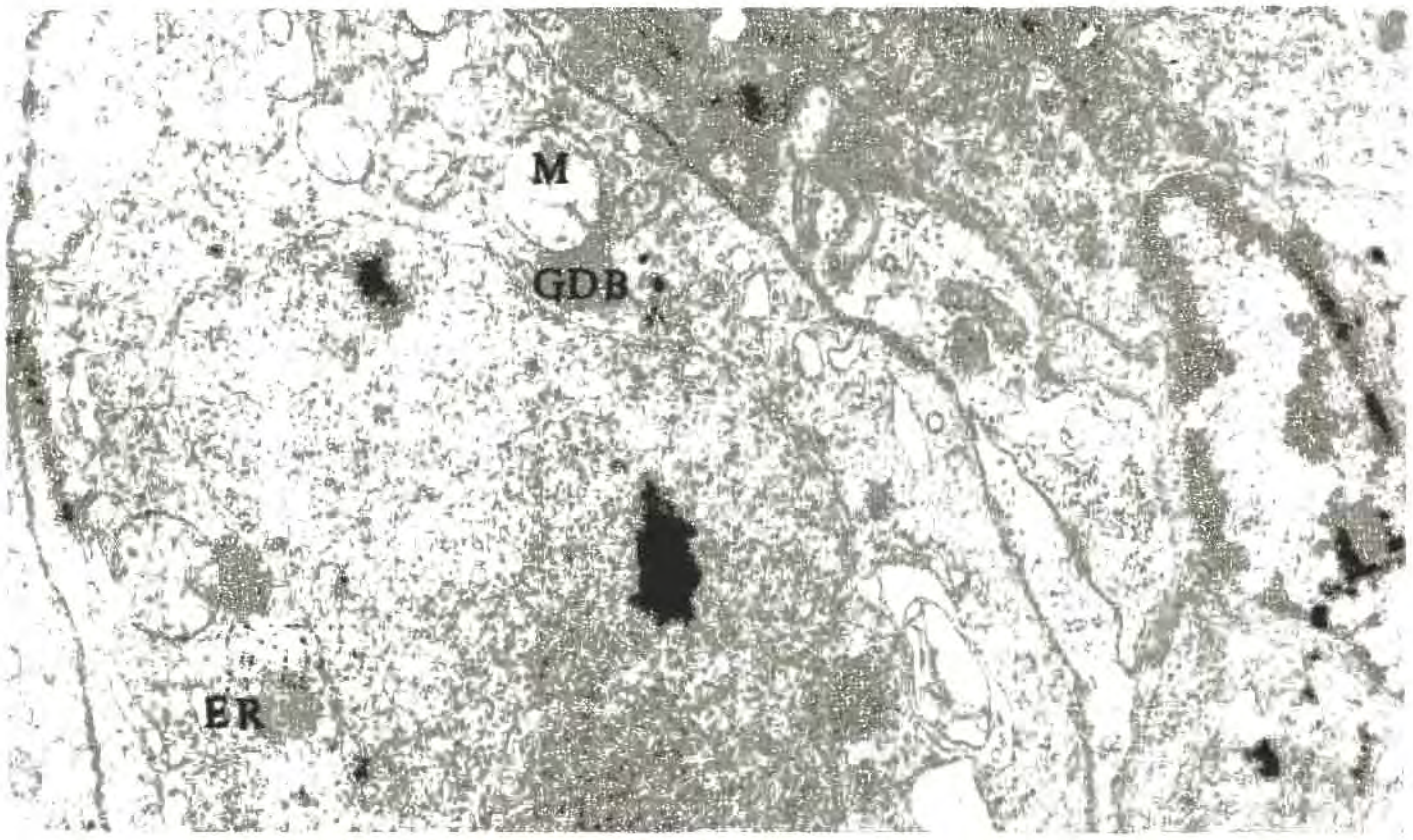
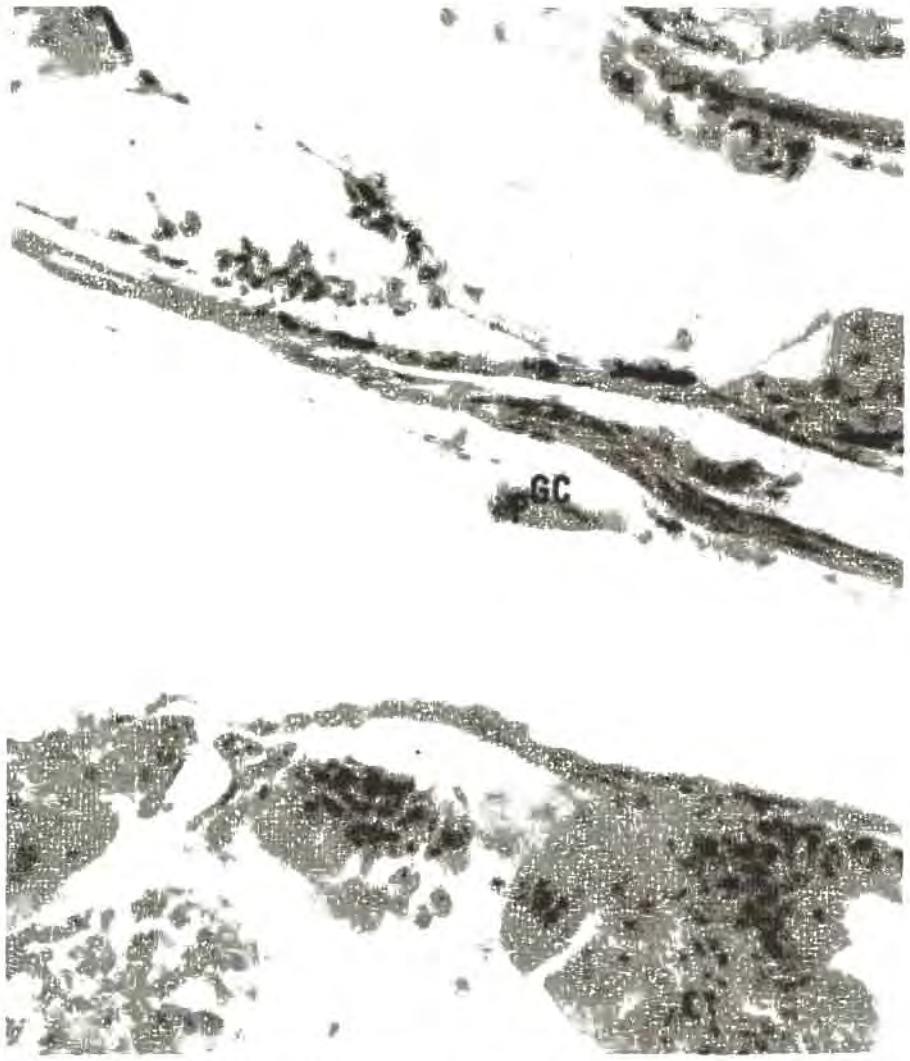


Fig. 2. Electron micrograph of a primordial germ cell (PGC). The cell and nuclear outlines are irregular. In the cytoplasm are large mitochondria (M), few dispersed ER lamellae (ER) and germinal dense bodies (GDB). x 30,000.



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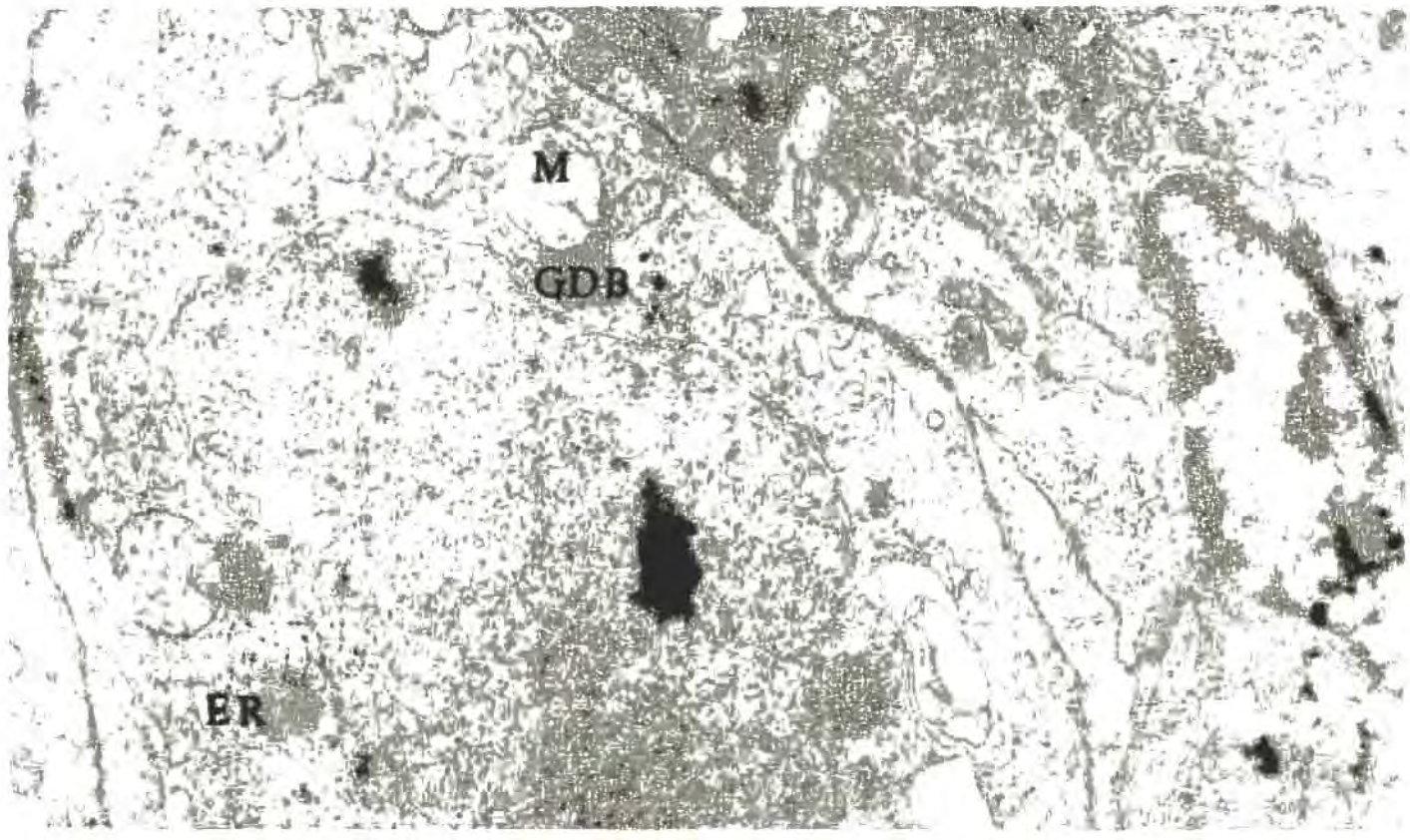

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Posthatch tilapia were kept in the same container until they reached the active feeding stage.

About 500 week-old fry were transferred to a "hapa" (nylon net enclosure) measuring 2 x 3 meters in one of the concrete fish ponds of the IFDR. Fish meal and "darak" (rice bran) were fed at about 4% body weight six times daily.

Histological techniques

From the time of hatching, 10 fry were fixed in Bouin's solution everyday for the first ten days and every two days for the succeeding weeks until the onset of gonadal maturity or the first appearance of fully formed sperm cells. For light microscopy, serial transverse sections of the region between the pronephros and the anus were stained with hematoxylin-eosin.

Based on the results of light microscopy, gonads of histologically significant developmental stages, were prepared for electron microscopy. The gonads were fixed in 2.5% glutaraldehyde, washed and soaked in phosphate buffer before post-fixation with 1% osmium tetroxide. Others were fixed in Karnovsky's glutaraldehyde-paraformaldehyde mixture in cacodylate buffer, washed and soaked in the same buffer before postfixation with osmium tetroxide. After dehydration in graded ethanol series, the specimens were embedded in Spurr's low viscosity epoxy resin. Ultrathin sections were mounted on uncoated grids and stained with uranyl acetate and lead citrate. These were examined with a Hitachi model H-300 electron microscope.

Results and Discussion

At the time of hatching

The primordial germ cells (PGC) at the time of hatching are in the gut endoderm, in the splanchnic mesoderm, or in the developing dorsal mesentery. The PGCs are clearly distinguishable from the somatic cells by their bigger size, larger nuclei, round to oval contour, and light cytoplasm (Fig. 1). In electron micrographs, the PGC has an irregular cellular and nuclear outline, a large heterochromatic to euchromatic nucleus, reticular nucleolus, few dispersed rough ER vesicles, and few to several large round mitochondria associated with germinal dense bodies (Fig. 2). The oval somatic cells flatten against the cell membrane of the PGC. The heterochromatic nucleus almost fills up the entire cell. The PGCs have a similar morphological appearance to the PGCs in *Oryzias latipes* described by Satoh (1974) characterized by the presence of germinal dense bodies interspersed with aggregations of mitochondria in the cytoplasm. The migratory pattern from endoderm to mesoderm to the genital ridges is similar to that of *Oryzias latipes* (Satoh and Egami, 1972) and *Cyprinus carpio* (Remojo, 1979).

Days 9-20 posthatching

At day 9, the PGCs establish themselves at the gonadal ridges (Fig. 3). Coloni-

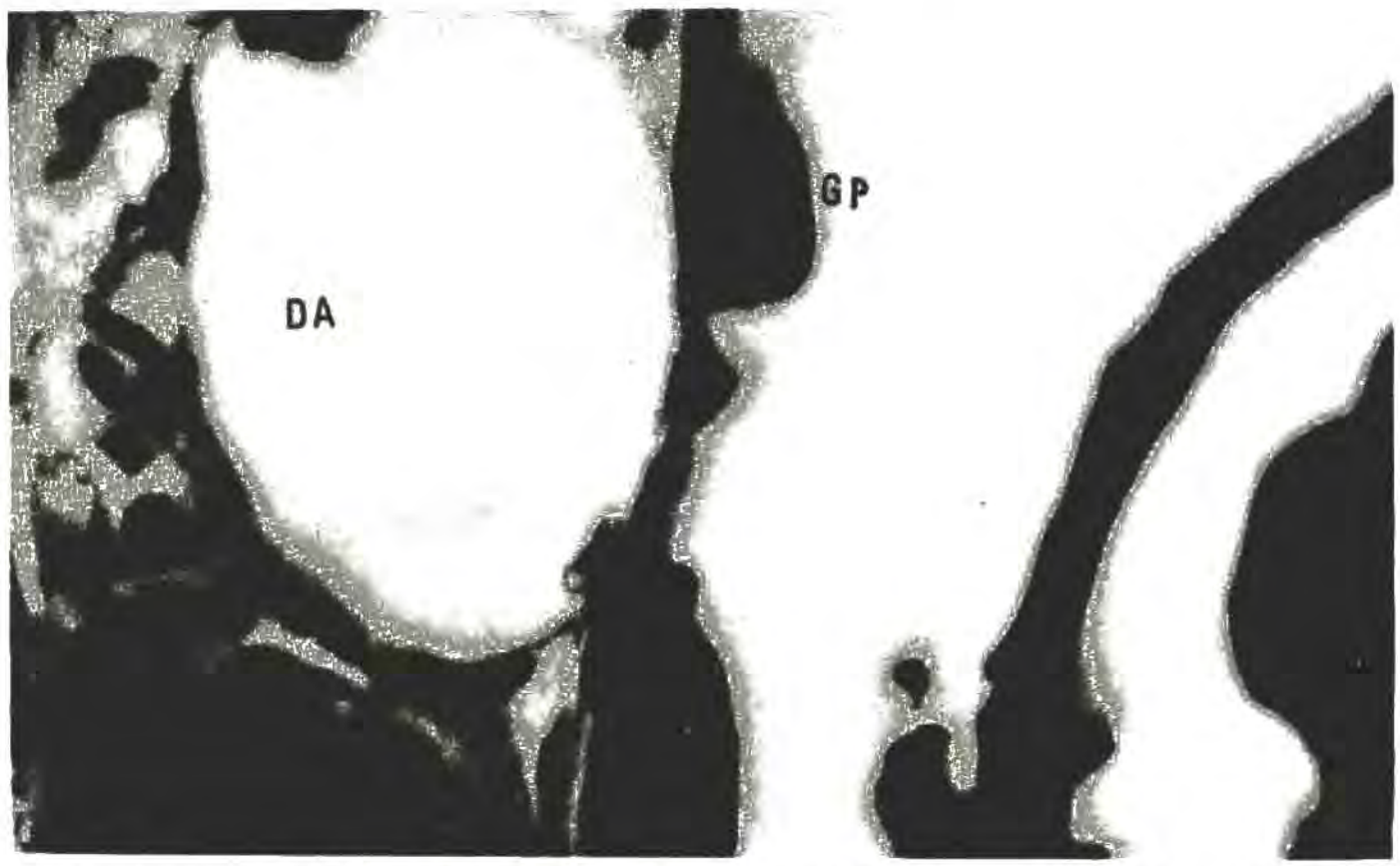

Fig. 3. The primodial germ cells (PGC) reach the gonadal ridges and form the gonad primordium (GP) which is composed of the PGCs and somatic cells (S). Hematoxylin-eosin x 400.

zation of the somatic tissues by PGCs with the formation of the paired gonadal primordia occurs at days 8-10 when the fry are about 11 mm. This coincides with the findings of Nakamura and Takahashi (1973) in *T. mossambica.* Eckstein and Spira (1965) observed it at day 8 in *T. aurea,* Boco (1977) at days 5-7 in *T. mossambica,* Yoshikawa and Oguri (1978) at day 6 in *T. zillii* and Brusle and Brusle (1978) at 8 months in *Mugil cephalus.* With the multiplication of the PGCs the gonadal anlagen enlarge. The gonad primordium consists of germ cells enveloped by somatic cells. The PGCs still look essentially the same as those of the newly-hatched fry. Fig. 4 shows a day 14 PGC. The cells have the same ultrastructural features as those of the PGCs of earlier developmental stages.

At about days 16-20, testicular differentiation occurs. In these gonads, germ cells are sparsely distributed, along the side facing the lateral peritoneal wall. Lumina (presumptive testocoel) are identified as splits in the stroma tissue (Fig. 5).

Electron micrographs show that there is already marked organization of the testis (Fig. 6). The somatic cells of the testis are elongated and irregular in shape, and show a notable aggregation near the mesogonium. The organization of the somatic cells bears a close resemblance to that of the day 40 testis. The nuclei are heterochromatic with irregular outlines. The nucleoli are granular. The cytoplasm contains mitochondria, scattered rough ER and vesicles.

Days 21-39

At this time, the testis still looks essentially the same as the 19-day old testis.

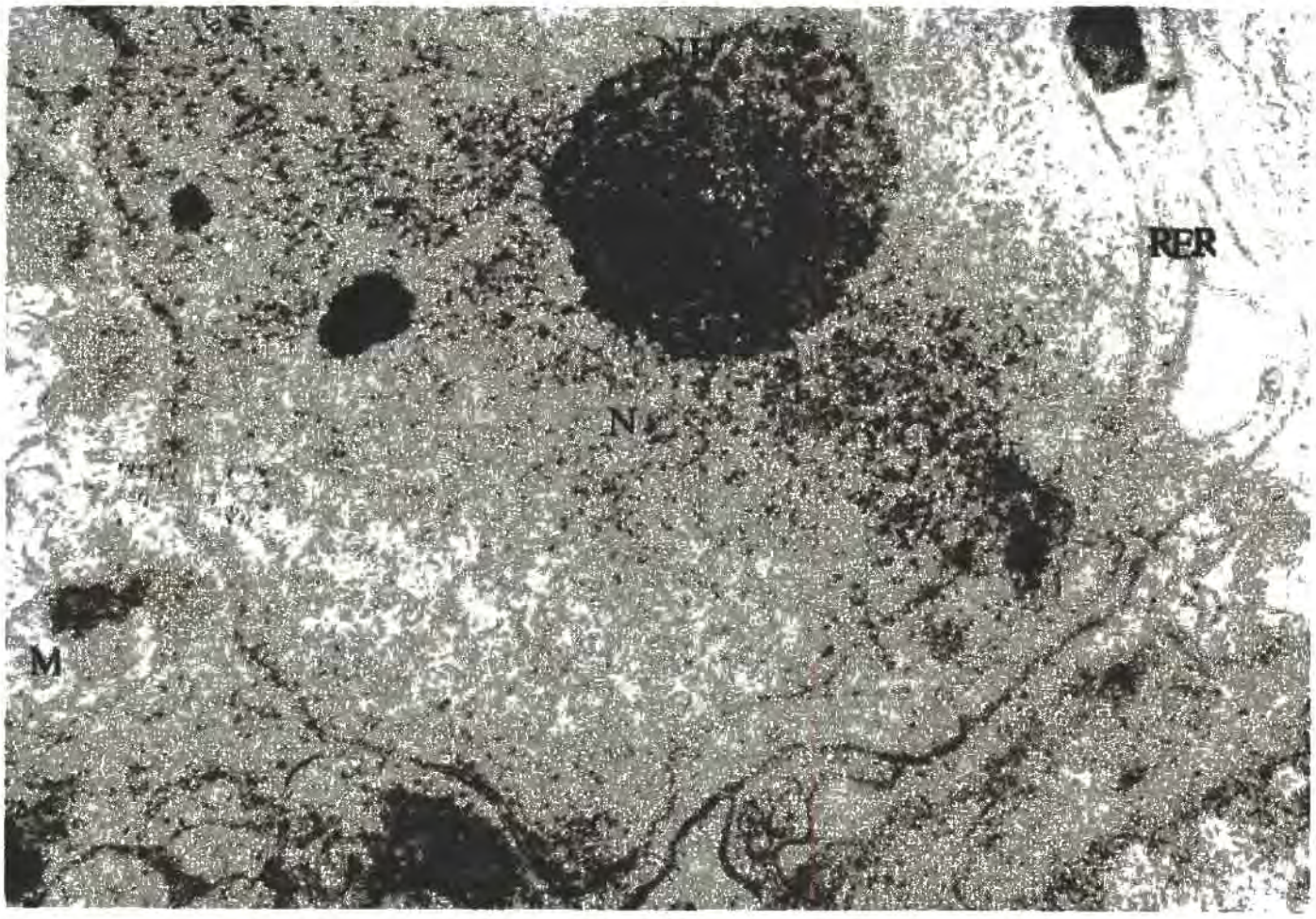

Fig. 4. Electron micrograph of day 14 PGC. N-nucleus, NU-nucleolus, RER-rough endoplasmic reticulum, M-mitochondria. x 14,000.

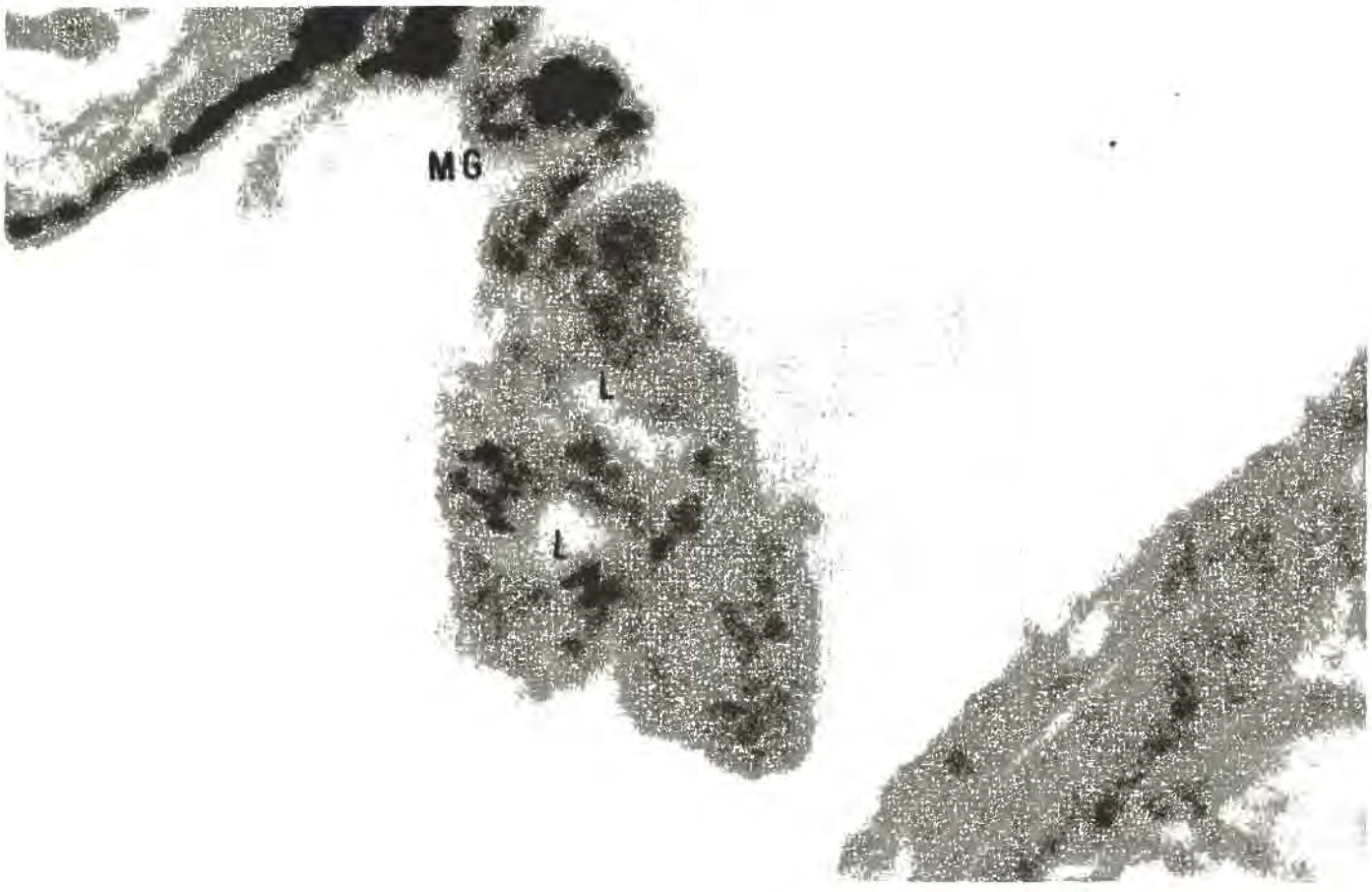

Fig. 5. The presumptive testis showing lumina (L) in the stroma tissue coincident with the efferent duct of the developing testis. MG-mesogonium, G-gut. Hematoxylin-eosin x 400.

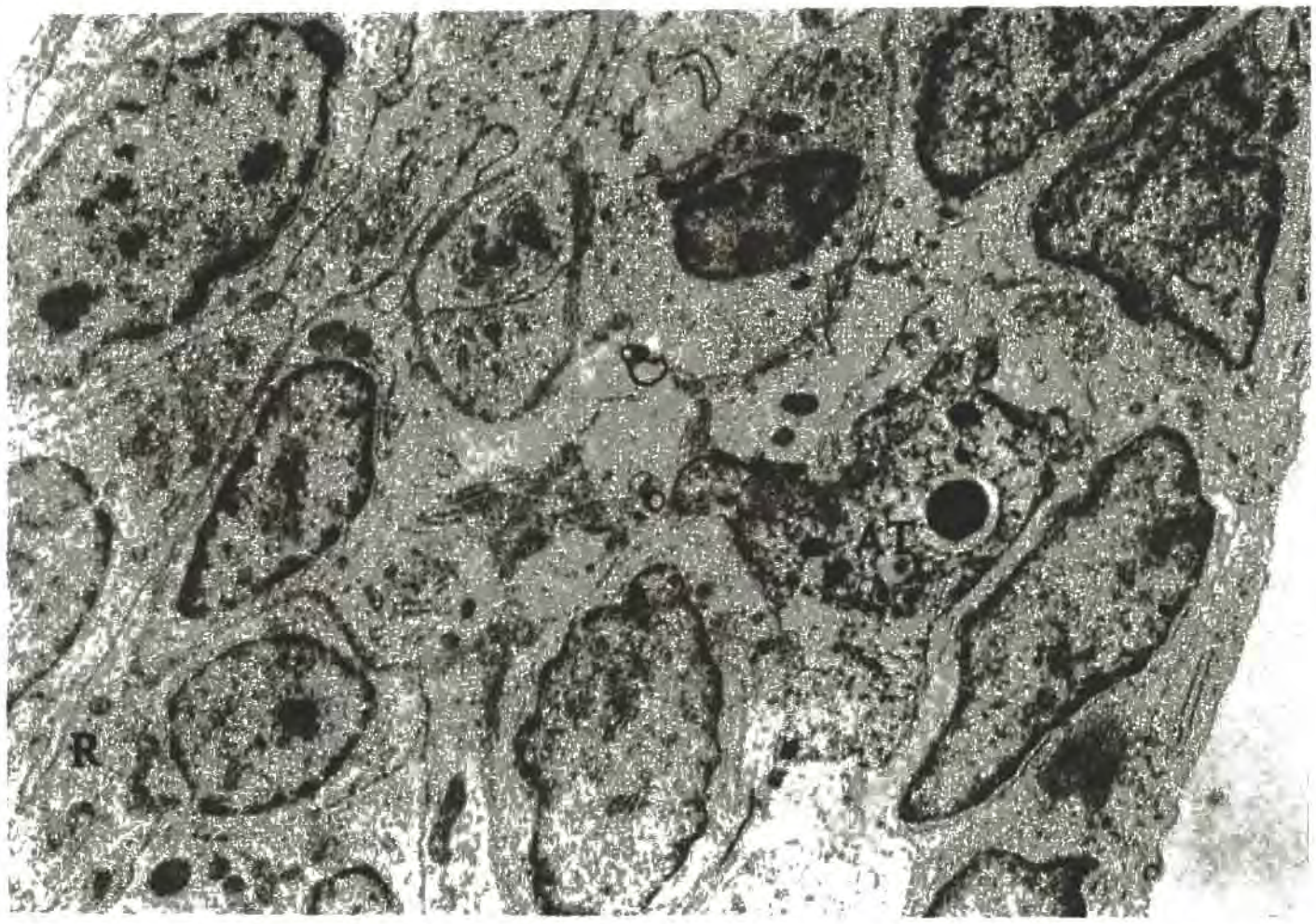

Fig. 6. Electron micrograph of a portion of the day 19 testis showing an aggregate of somatic cells with irregular cell and nuclear shapes, heterochromatic nucleus, dispersed ribosomes (R), vesicles and RER lamellae. AT-atretic cell. x 10,000.

Days 40-73

At about day 40, seminiferous tubules are more readily recognized in the testis (Fig. 7). Averaging 600 μm, the tubules are separated by myoid boundary cells. They are composed of large PGCs, spermatogenic and somatic cells (presumptive myoid boundary cells, Sertoli cells, interstitial cells). The PGCs have a very irregular cell and nuclear shape. In the cytoplasm are a few large mitochondria and some dispersed ER lamellae (Fig. 8).

The spermatogonia adjacent to the irregularly-shaped myoid boundary cells conform to the contour of the network. The spermatogonia farther away from the testis periphery where the myoid boundary cells abound have more or less regular cell outlines (Fig. 9). The heterochromatic nucleus is spherical to oval, and contains a prominent granular nucleolus. In the cytoplasm are several mitochondria, rough ER, Golgi bodies and ribosomes.

The somatic cells are much smaller than the germ cells. The myoid boundary cell body shows an irregular, heterochromatic nucleus. The interstitial cells are polygonal with heterochromatic, irregular nucleus. In the cytoplasm are several mitochondria, vesicles, rough ER and ribosomes (Fig. 10).

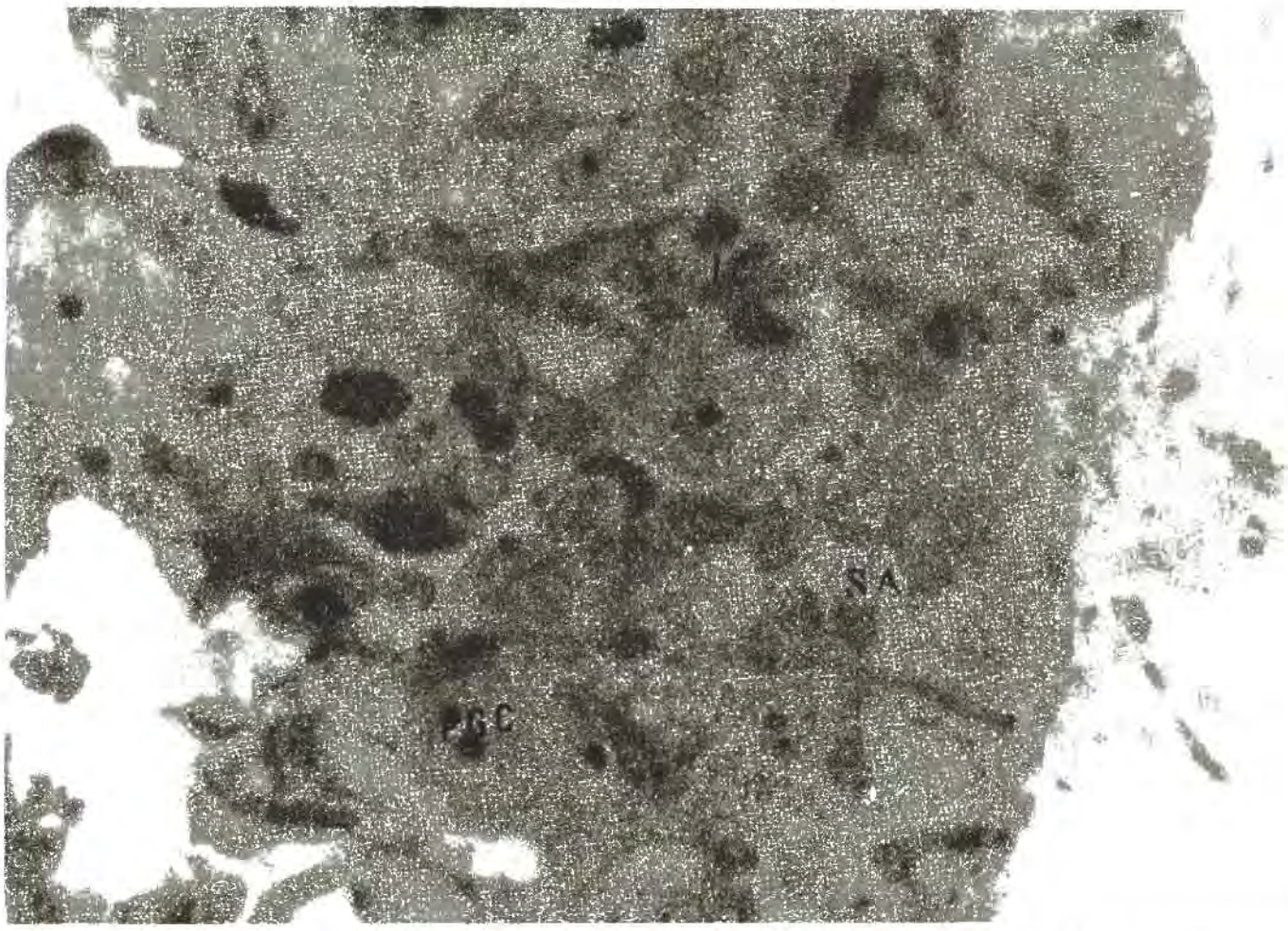

Fig. 7. The seminiferous tubules of the day 40 testis, IC-interstitial cell, SA-spermatogonia, PGC-primordial germ cell. x 1,000.

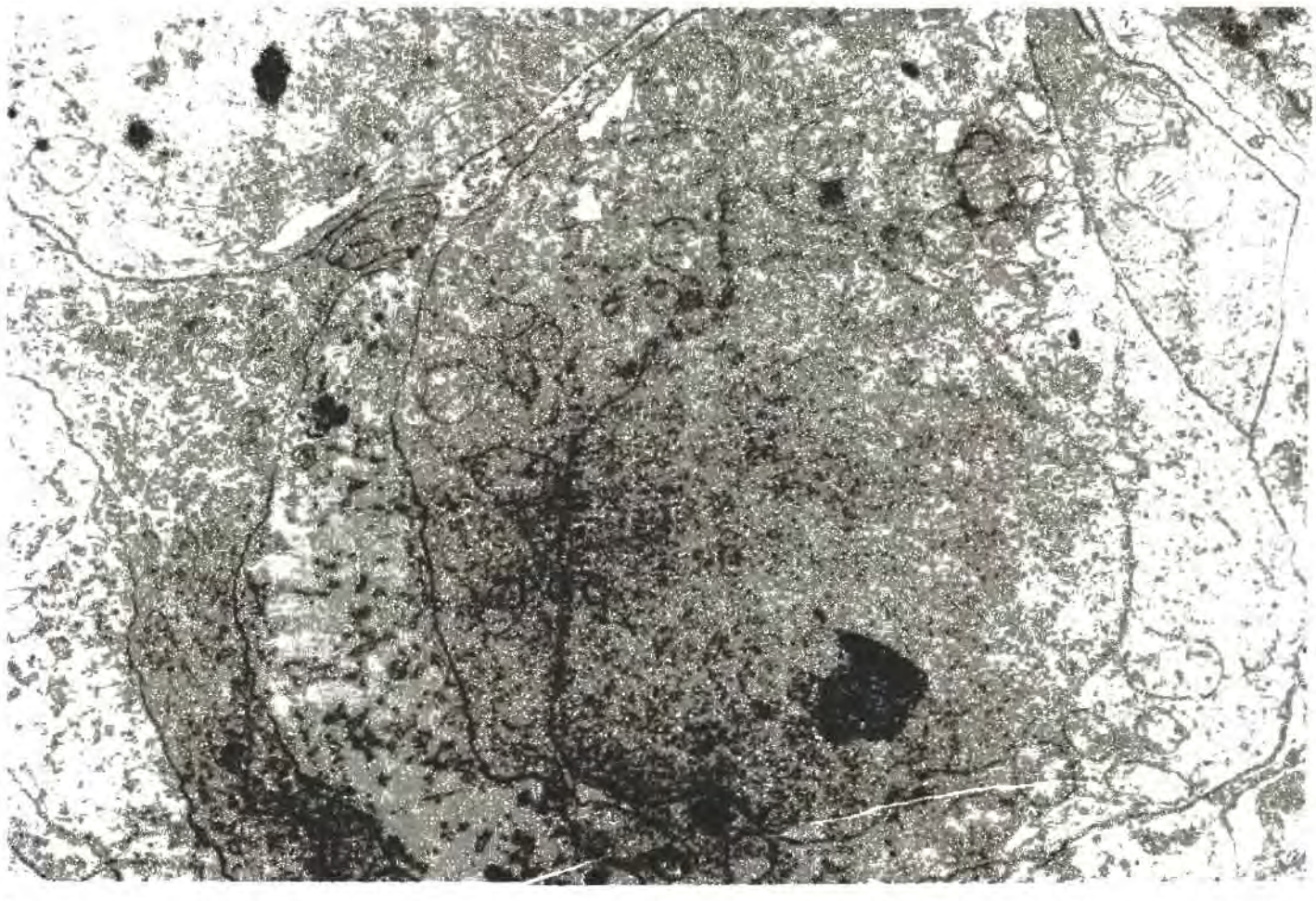

Fig. 8. Electron micrograph of a primordial germ cell at day 40. The cell and nuclear shapes are irregular. Germinal dense bodies (GDB) are present. x 13,600.

Fig. 9. Spermatogonia far from the myoid boundary cells have a more or less regular cell outline. VA-vacuole, M-mitochondria. x 13,300.

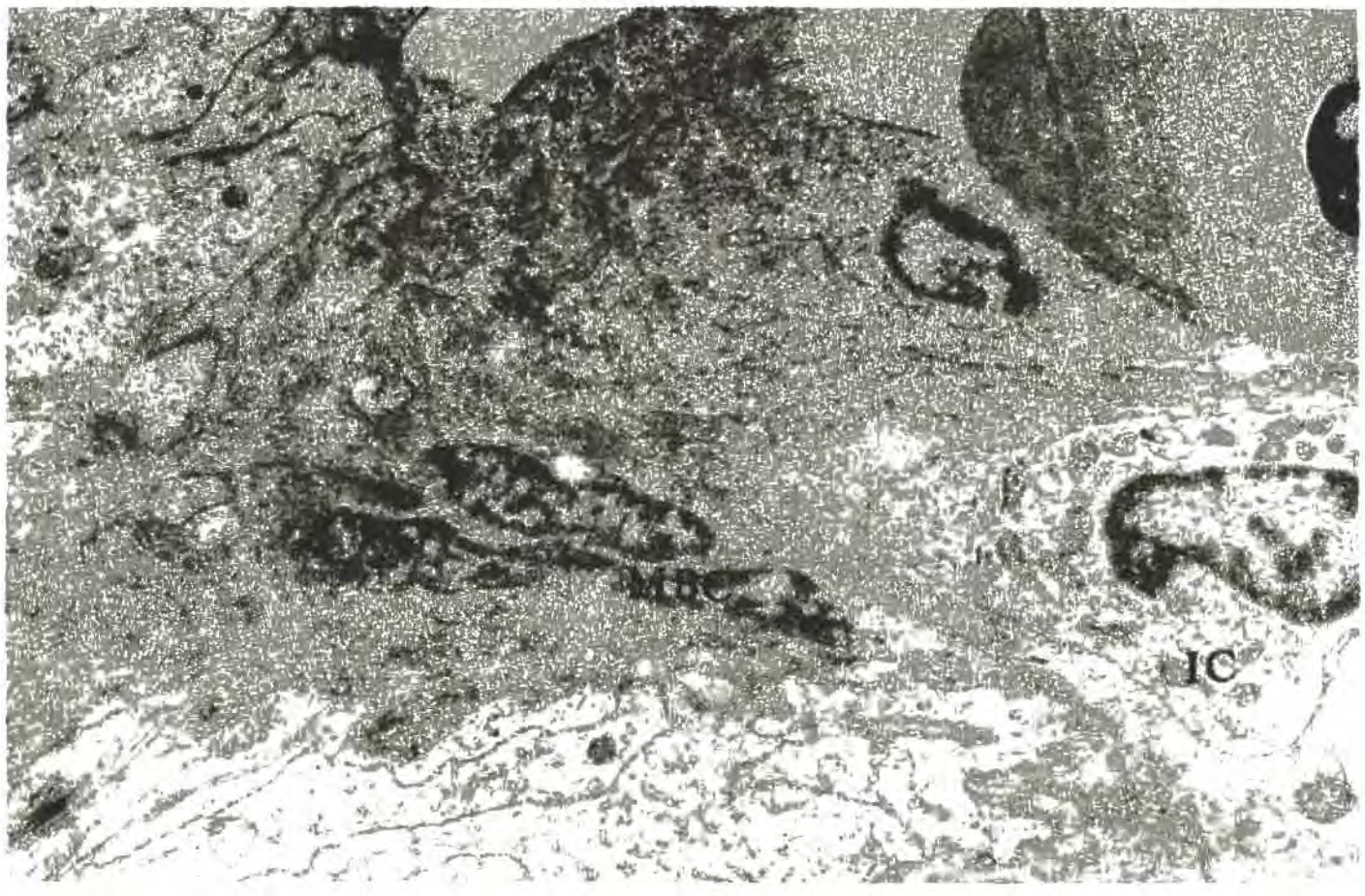

Fig. 10. Electron micrograph of the myoid boundary cells (MBC), and the interstitial cells (IC) of the day 40 testis. MBCs have cytoplasm extensions that serve as outer boundary of the seminiferous tubules. x 13,300.

By day 73 the testis is well into very active spermatogenesis. The cysts formed by the repeated division of gonia have become larger. With each division the cell sizes decrease and the cell group becomes larger. Chromatin of secondary gonia are highly condensed (Fig. 11). Mitochondria with parallel cristae are curved. Golgi bodies and rough ER are well-developed and ribosomes are scattered in the cytoplasm. The large round, heterochromatic nucleus almost fills up the entire cell. The primary spermatocytes, larger than the spermatogonia, are in various stages of meiotic prophase. Fig. 12 shows primary spermatocytes in pachytene stage. Chromosomes in synaptonemal complex can be easily recognized. In the cytoplasm are scattered vesicles, abundant free ribosomes, a few round to oval mitochondria and some rough ER lamellae. At no time after 66 days is a pure population of a single germ cell type present throughout the testis.

Days 88-100

The testis at this stage contains numerous large tubules filled with cells in varying stages of spermatogenesis (Figs. 13, 14, 15). Sperms fill the efferent ducts and main ducts ready for spermiation (Figs. 16, 17).

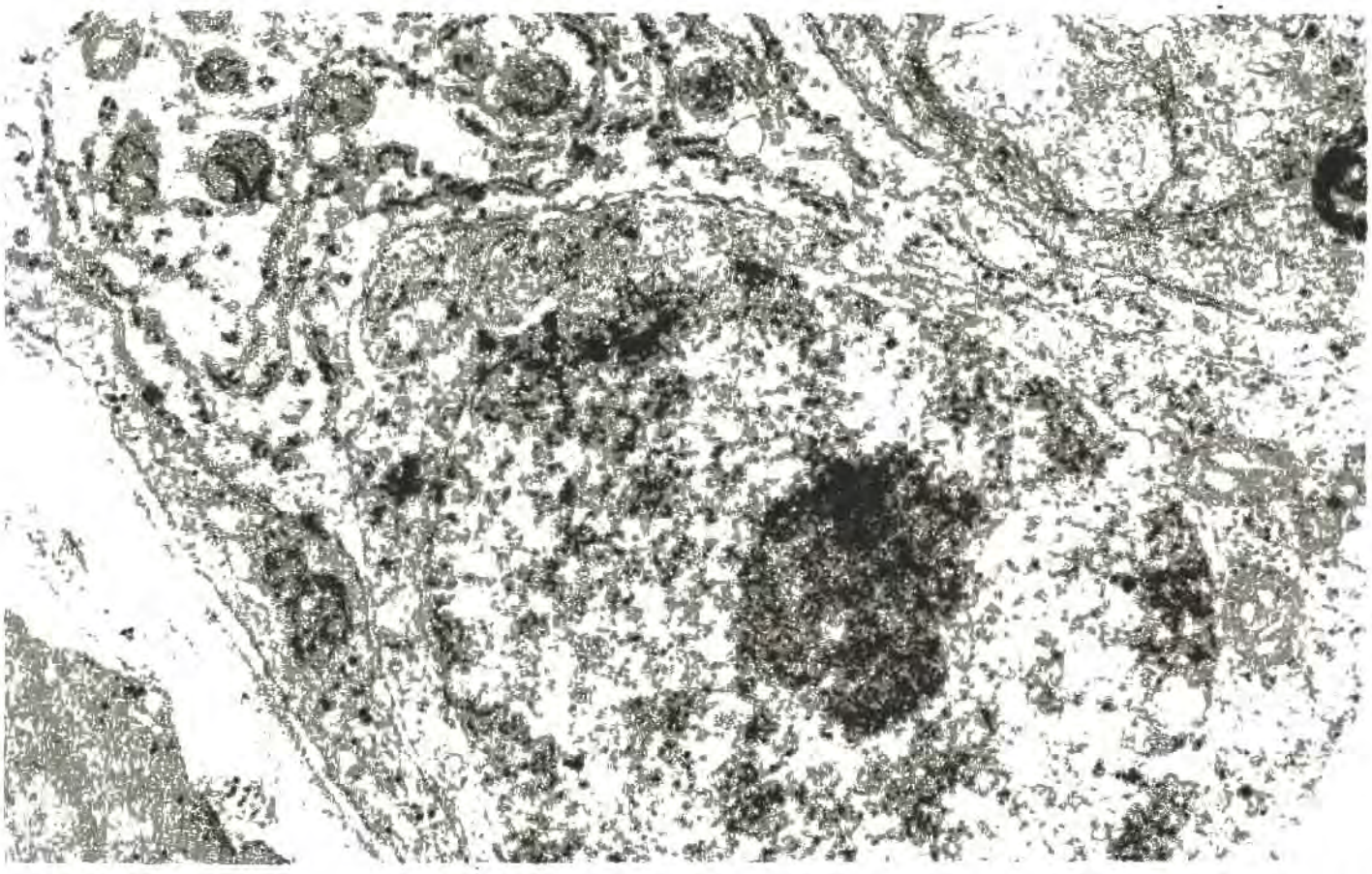

Fig. 11. Electron micrograph of spermatogonia at day 66. The cells have become smaller by the repeated division of a single spermatogonium. Mitochondria (M) with parallel cristae are observed. x 14,000.

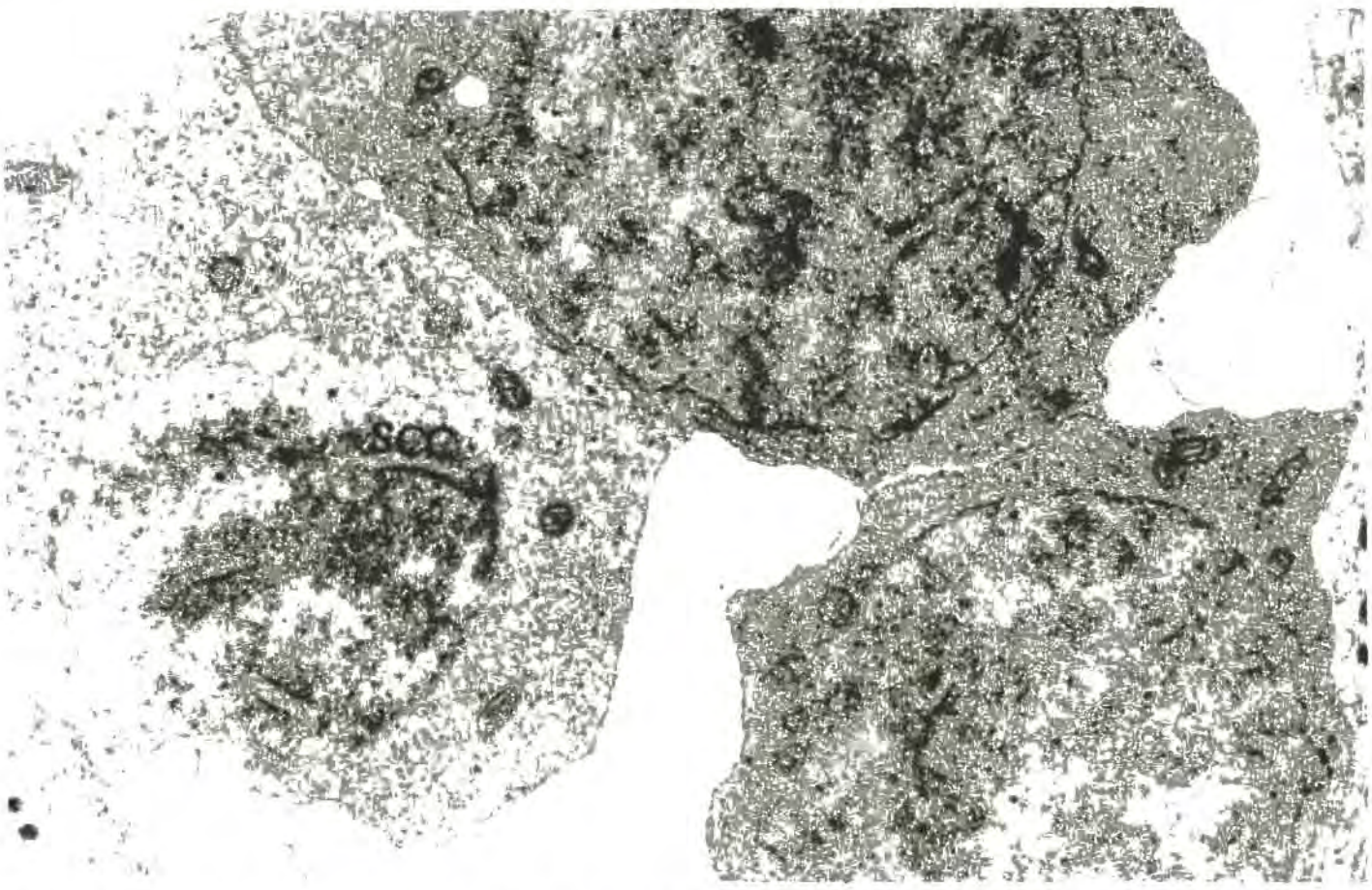

Fig. 12. Primary spermatocytes of the day 66 testis. The synaptonemal complex configuration (SCC) is clearly visible, the tripartite structure consisting in longitudinal section of two thick lateral elements and a fine central element running between them. The width of the complex is approximately 150 nm and the central element is about 10 nm in thickness. x 20,000.

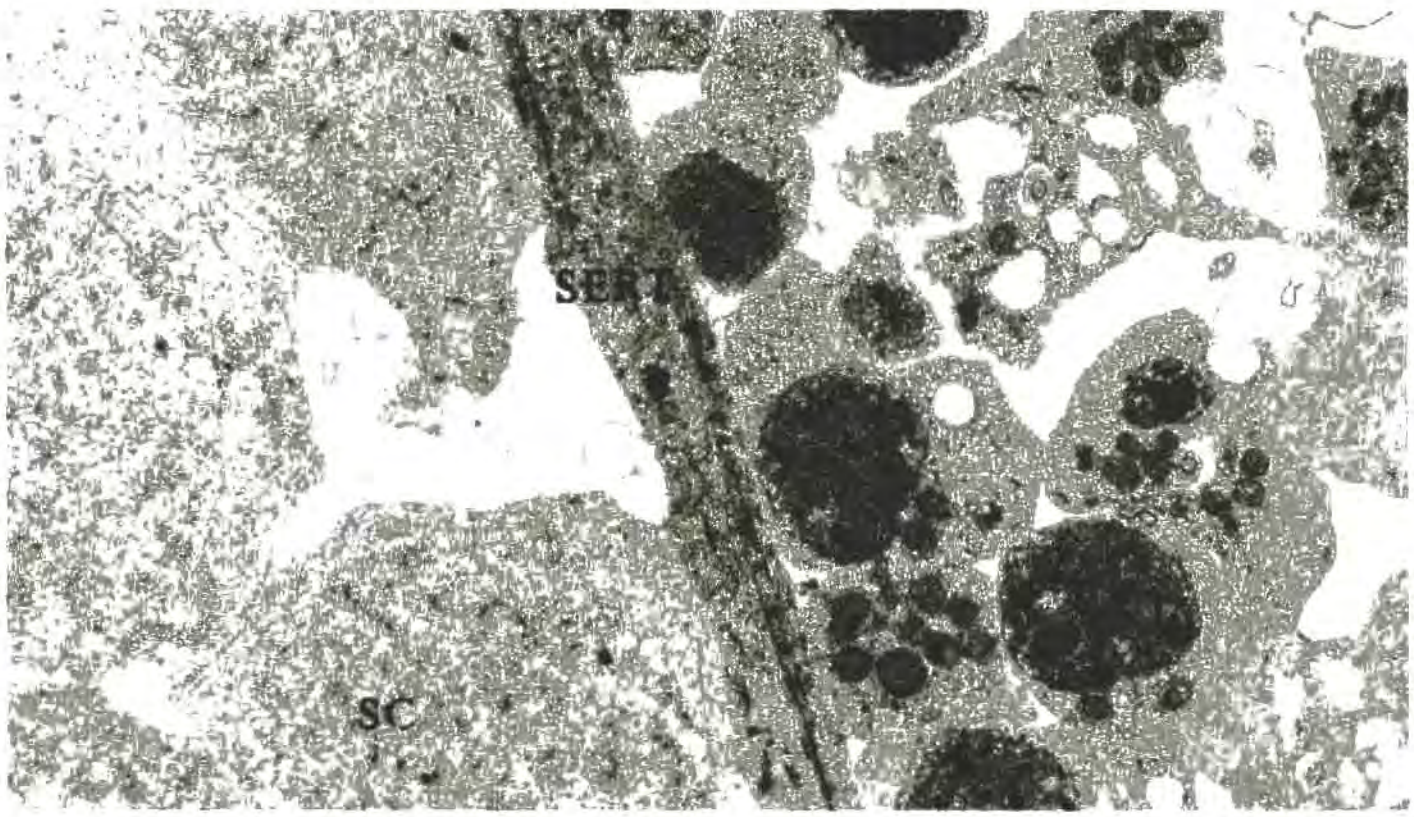

Fig. 13. Electron micrograph of primary spermatocytes (SC), spermatids (ST) and portion of a Sertoli cell (SERT). The primary spermatocytes are in zygotene. Mitochondria are proliferating to localize in the sperm midpiece during spermiogenesis. M-mitochondria, F-flagella. x 10,000.

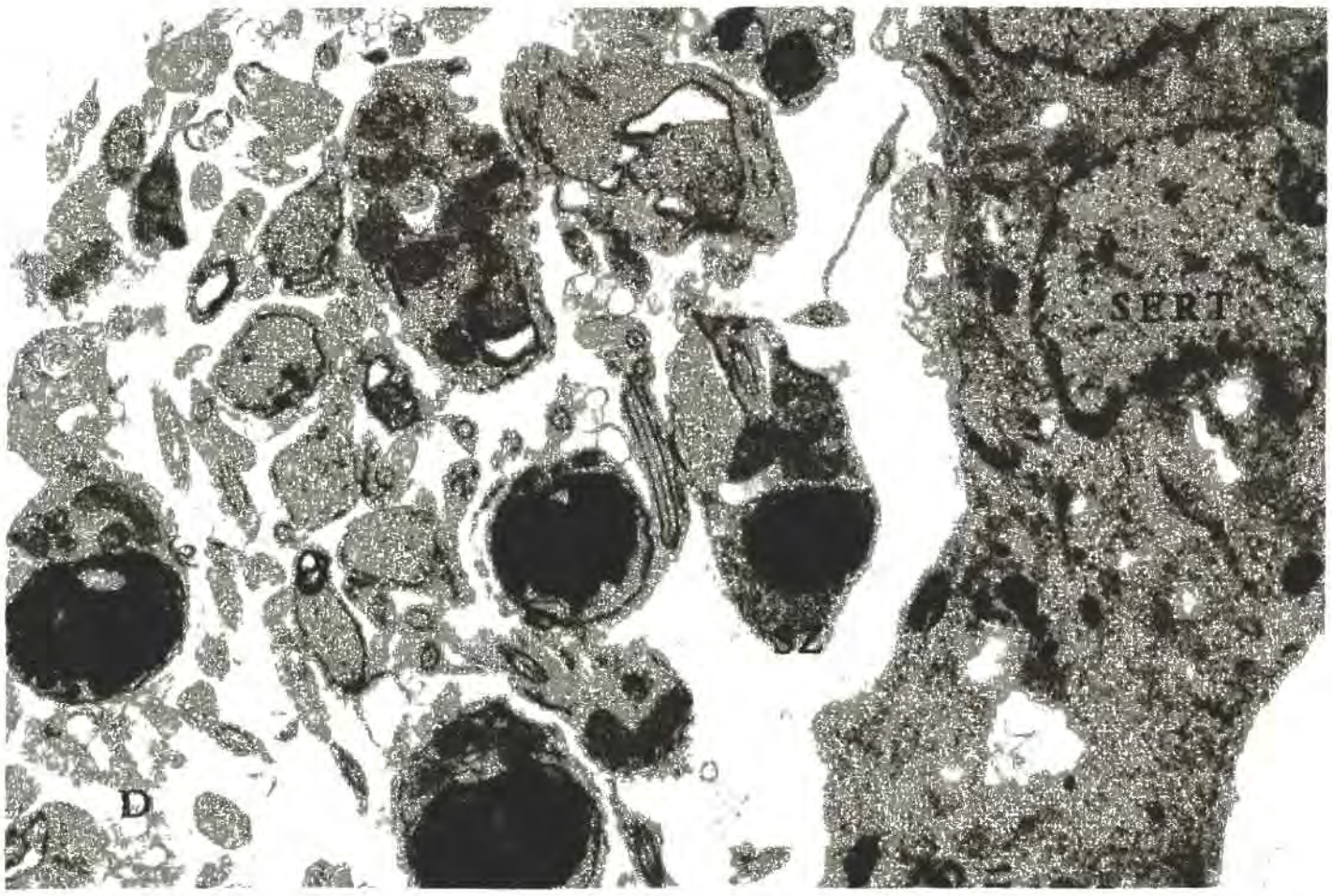

Fig. 14. Electron micrograph of a cluster of sperm cells (SZ) showing their condensed chromatin. Sertoli cells (SERT) line the efferent ducts. D-debris of cast-off cytoplasm of sperm. x 10,000.

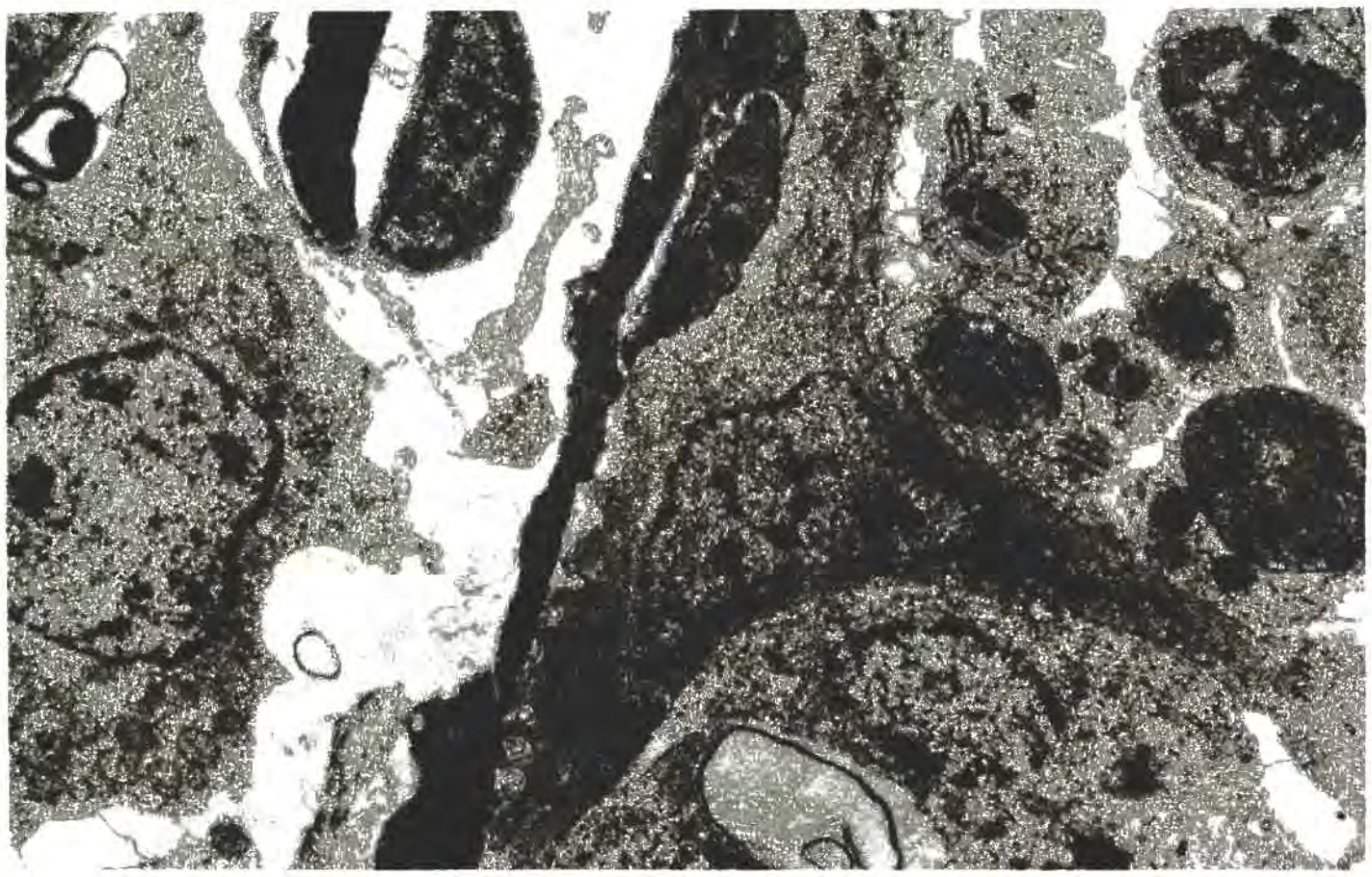

Fig. 15. Electron micrograph of a portion of a seminiferous tubule showing a Sertoli cell separating the cyst of spermatids in spermiogenesis from the cyst of spermatocytes. The myoid boundary cell lies outer to the Sertoli cell. AL-annulated lamellae. x 10.000.

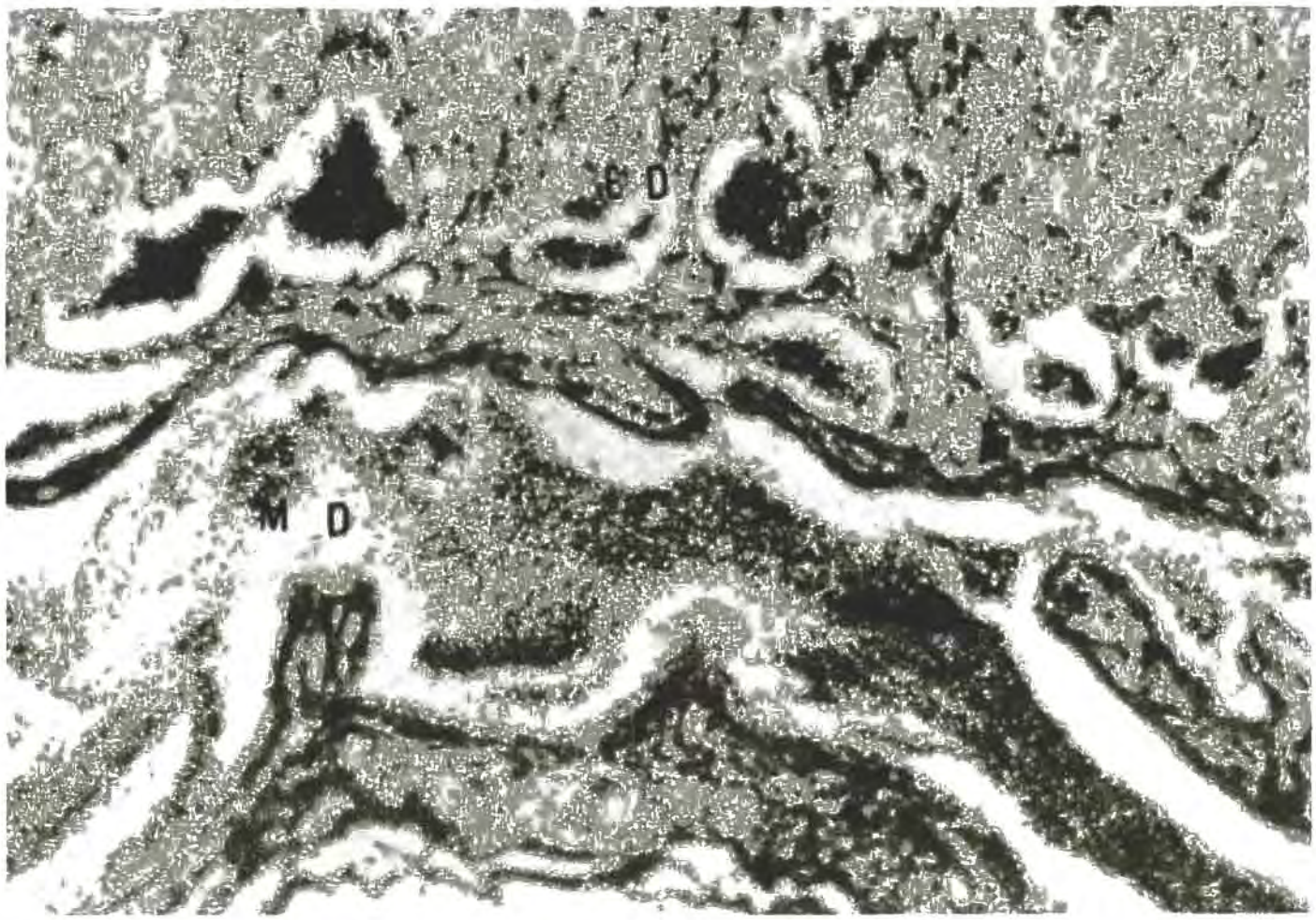

Fig. 16. The day 88 testis contains an abundance of spermatozoa (SZ) in the efferent ducts (ED) and main ducts (MD) of zone I. Hematoxylin-eosin. x 100.

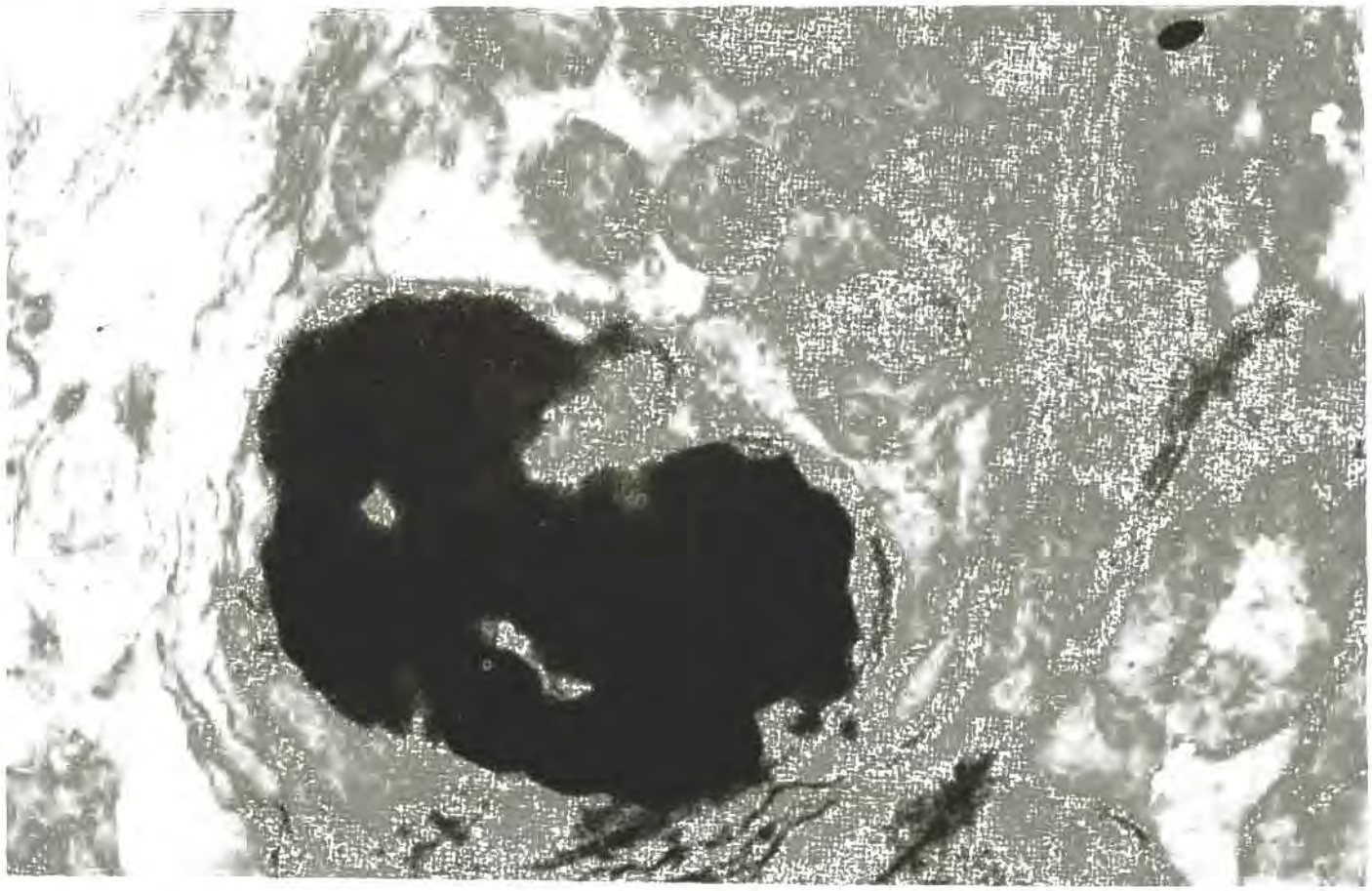

Fig. 17. A fully differentiated sperm. x 15,000.

Summary

The ultrastructure of spermatogenesis in *T. nilotica* is basically similar to that of other male vertebrates (Leeson and Leeson, 1985). The first batch of fully differentiated sperm formed during ontogeny are ready for spermiation by 88-100 days posthatching.

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