Spermiogenesis in the rainbow trout (*Salmo gairdneri*)

An ultrastructural study

Roland Billard
I.N.R.A., Laboratoire de Physiologie des Poissons, Rennes, France

Summary. In an ultrastructural study on the spermiogenesis of the rainbow trout (*Salmo gairdneri* R.) four spermatogenetic stages were identified. In young round spermatids, the nuclear chromatin was first heterogeneous (euchromatin and heterochromatin). Subsequently, it became more homogeneous and started to condense in the form of coarse granules and fibers and then into fibrils associated in ribbon-like elements which eventually partly fused together. During early spermiogenesis, a juxtanuclear vacuole appeared in the area where the nuclear envelope was specialized due to condensation of material between the two envelopes and a slight accumulation of nuclear material. This area was finally located in the anterior part of spermatids and spermatozoa; it probably plays a role during fertilization. A flagellar rootlet appeared early in spermiogenesis; it may play a role in the attachment of the flagellum to the nucleus since it persisted until the centriolar complex was definitively fixed in the implantation fossa. The flagellum did not display a plasma membrane and was first located in the cytoplasm, but when it was later extruded from the cell, it acquired a membrane. The cytoplasm was rich in ribosomes (free or in small groups) but poor in membranous organelles. The few mitochondria polarized around the centriolar complex were finally organized into an annular mid-piece. The spermatids remained connected by intercellular bridges until the end of spermiogenesis. The complexity of trout spermiogenesis is intermediate between that in poecilids and that in carp and pike, which have very simple spermatozoa. The role of the material from the nucleus and the cytoplasm reaching the Sertoli cell in the control of spermatogenesis has been discussed.

Key words: Spermiogenesis – Rainbow trout – Ultrastructure – Spermatids – Chromatin

Send offprint requests to: Dr. Roland Billard, I.N.R.A. Laboratoire de Physiologie des Poissons, Campus de Beaulieu, F-35042 Rennes, France
The formation of spermatozoa during the last phase of spermatogenesis, and especially the transformation of nuclear protein (Alfert 1956; Felix 1960; Dixon and Smith 1968; Davies et al. 1976), have been thoroughly studied by biochemical means. Some of the rare, detailed morphological studies that exist, however, indicate that spermiogenesis in salmonids has several particular characteristics.

An ultrastructural and morphometric study by Zirkin (1975) has shown that the process of chromatin condensation is complex. Important transformations, such as the temporary presence of flagellar rootlets, also affect the cytoplasmic organelles during the morphogenesis of spermatozoa (Billard 1972). No comprehensive study has been carried out on the entire process of spermiogenesis in one species. The present investigation is an approach to such a study in the rainbow trout (Salmo gairdneri).

Materials and methods

Adult male rainbow trout were obtained from various trout farms in the Paris area or from the trout breeding facility at our laboratory in Jouy-en-Josas; these fish had already had at least one reproductive cycle.

They were decapitated between September and February after anesthesia in a 2% solution of MS222. Pieces from the middle of the testis were sampled and fixed for 1 h in a 3.3% glutaraldehyde solution in 0.1 M phosphate buffer at pH 7.25 and then fixed for 1 h in a 2% solution of osmium tetroxide in the same buffer; in some cases, the pieces were fixed directly in the osmium solution. After embedding in Epon, the pieces were cut into sections and stained with uranyl acetate and lead citrate for 5 min before they were covered with a carbon film for observation with a Siemens EM I electron microscope.

Results

Spermiogenesis in trout is divided into four stages which are described below and summarized in Table 1:

**Stage 1.** Immediately after the last maturation division of secondary spermatocytes, the round spermatids grouped in cysts are interconnected by intercellular bridges. In the nucleus, which remains spherical, granular filamentous chromatin is irregularly distributed (Figs. 1–3), with scattered perichromatin granules (Figs. 1–4) and dense bodies (Fig. 3) which might represent nucleolar remnants. The flagellum is formed in the cytoplasm early in this stage and remains inside the cytoplasm. The centriolar complex is surrounded by small vesicles (Fig. 1) and becomes connected to the nucleus by a flagellar rootlet (Fig. 2, Table 1). The membranous structures (Golgi apparatus and endoplasmic reticulum) of the cytoplasm are poorly developed; ribosomes are usually found free in the cytoplasm. Mitochondria with a dense matrix are polarized around the centriolar complex early in spermiogenesis (Fig. 2).

Another peculiarity of early spermiogenesis is the presence of a small vacuole in the anterior part of the nucleus in close contact with the nuclear envelope which is more dense (Fig. 3). This vacuole expands slightly (Fig. 4)