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Scanning Electron Microscopy of the Rainbow Trout (*Salmo gairdneri* Richardson) Spermatozoon

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ABSTRACT

The scanning electron microscope was used to determine the morphology of the rainbow trout (*Salmo gairdneri* Richardson) spermatozoon. The spermatozoon is approximately 32 μm long and consists of a head, mitochondrial collar, and flagellum. The head is elongated and somewhat flattened. It has an antero-posterior length of 3.1 μm and a maximum diameter of 1.6 to 2.2 μm . Mean antero-posterior length of the mitochondrial collar is 0.8 μm . The collar encircles the flagellum but is separated from it. The flagellum ranges in length from 26 to 31 μm and is divided into a principal piece and end piece. Cytoplasmic vesicles commonly are found in the anterior region of the flagellum.

INTRODUCTION

Scanning electron microscopy is a relatively new technique that yields three-dimensional views of objects at high magnifications by bombarding the specimen with a moving beam of electrons. Although highest magnification is less than that obtained by transmission electron microscopy, the three-dimensional representations of specimens make scanning electron microscopy a valuable adjunct to studies of the morphology of biological specimens.

The scanning electron microscope has been used to study human, hamster, bull, rabbit, ram, monkey, boar, and turkey spermatozoa (see, for example, Dott 1969, Fujita et al. 1969, Gould et al. 1971, Zaneveld et al. 1971, Hafez and Kanagawa 1973, Yasuda and Tanimura 1974, Marquez and Ogasawara 1975). However, a review of the literature has failed to disclose scanning electron microscope studies of fish sperm cells. The purpose of this report is to describe the morphology of the rainbow trout (*Salmo gairdneri* Richardson) spermatozoon as revealed by the scanning electron microscope (SEM) and to coordinate the results with transmission electron microscope (TEM) findings.

METHODS AND MATERIALS

Milt was collected by stripping ripe rainbow trout. Care was taken to avoid contaminating the samples with water, urine, or feces. Specimens were fixed in 4% glutaraldehyde in Millonig's phosphate buffer for 8 hours, then centrifuged, and the sediment was stored in s-collidine buffer (pH 7.38) until further processing was performed.

For SEM studies, smears were made on microscope slide fragments that had been coated with a 0.5% gelatin solution and allowed to dry. The smears were dehydrated in an ethanol series, then processed through absolute ethanol-amyl acetate solutions containing increasing concentrations of amyl acetate, and finally were rinsed twice with 100% amyl acetate. Samples were flooded with amyl acetate to prevent air drying and were transferred to a critical-point drying apparatus where they were dried with carbon dioxide. The dried specimens were coated with carbon and 60:40 gold palladium and were examined on a Cambridge Stereoscan 600 at accelerating voltages of 15 and 25 kV.

For TEM studies, testes from ripe males were excised, diced into blocks of about 1 mm³, then fixed in glutaraldehyde and stored as described above. Specimens were postfixed in 2% osmium tetroxide, dehydrated rapidly in a graded methanol series with extended soaking in absolute methanol, and embedded routinely in epoxy-resin. Thin sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome and supported on naked 300 mesh copper grids. These were stained sequentially with potassium permanganate, uranyl acetate, and lead citrate (Soloff 1973), and were examined with an Hitachi model HU-11B electron microscope at an accelerating voltage of 75 kV.

RESULTS

The spermatozoon of rainbow trout is approximately 32 μm long. It has a head and flagellum with no clearly differentiated neck or midpiece (Fig. 1). The head is somewhat elongated and appears to be flattened on the substrate. In some sperm cells, a mitochondrial collar or lobe can be identified near the posterior region of the head (Fig. 2). The mean antero-posterior length of the head is 3.1 μm measured from its anterior tip to the ridge demarcating the mitochondrial collar. Maximum diameter of the head ranges from 1.6 to 2.2 μm . Mean antero-posterior length of the mitochondrial collar is 0.8 μm . The collar encircles the flagellum and is separated from it by the cytoplasmic canal (Fig. 3). Some observations of sperm positioned at unusual angles introduce the possibility that the collar may not encircle the flagellum completely. Analysis of both SEM and TEM samples failed to show the presence of an acrosome.

The flagellum ranges in length from 26 to 31 μm and is divided into a principal piece and end piece (Fig. 1). The flagellum diameter appears to be constant throughout the principal piece, but narrows sharply at the transition to the end piece. One or more cytoplasmic vesicles are observed commonly at the anterior region of the flagellum (Figs. 1, 3).

TEM studies (Fig. 4) confirm the presence of mitochondria in the collar. The head is composed of a dense, coarse, granular material and is covered by a nuclear envelope. Separation of the mitochondrial collar from the flagellum by the cytoplasmic canal is evident in sectioned material.

DISCUSSION

Using the transmission electron microscope, Billard (1969) studied the sperm ultrastructure of various species of fishes including rainbow trout (*Salmo gairdneri*) and brown trout *Salmo trutta fario* (Linnaeus). Our observations conform with his and integrate the surface morphology of the sperm cells with their ultrastructure. Our head measurements are slightly greater than those reported by Billard but the difference may be that his represent average measurements made on both species. The dimensions of rainbow trout spermatozoa are in general agreement with those reported for other species of Salmonidae (see Ginzburg 1972 for a summary of these investigations).

The apparent absence of an acrosome agrees with TEM findings reported for carp (Fujimura et al. 1956), guppy (Porte and Follenius 1960), midshipmen (Stanley 1965), goldfish (Fribourgh et al. 1970), and channel catfish (Jaspers 1972). It has been suggested that the absence of an acrosome may be related to the presence of a micropyle in the eggs of teleost fishes.

Morphology of the mitochondrial collar, as revealed by SEM, supports deductions made from TEM studies (Billard 1969, Nicander

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1969, Stanley 1969, Fribourgh et al. 1970, Jaspers 1972). It has been suggested that the morphology of the collar (also called cytoplasmic collar or lobe) may be associated with fertilization. Low collars are present if fertilization is external and high collars are found in viviparous species (Porte and Follenius 1960, Dadone and Narbaitz 1967, Stanley 1969). Separation of the flagellum from the mitochondrial collar by a cytoplasmic canal has been reported for other species with external fertilization and compares with the structure of mammalian spermatids (Billard 1969, Nicander 1969, Fribourgh et al. 1970, Jaspers 1972).

Our study shows the presence of cytoplasmic vesicles in the anterior region of the flagellum. This finding agrees with TEM findings in rainbow and brown trout (Billard 1969). A discussion of the proposed nature and relationships of this structure is given by Ginzburg (1972).

Demonstration of a well-defined end piece in rainbow trout agrees with observations reported for lake trout (Ginzburg 1972). Tails of the spermatozoa of some fishes gradually thin toward the end (carp, guppy) in contrast to the flagella of bream sperm that remain the same diameter to the tip (Ginzburg 1972).

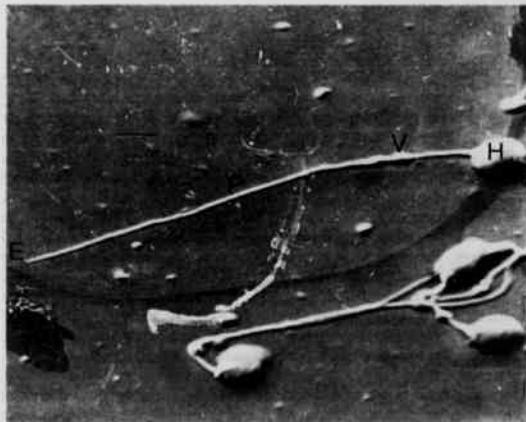


Figure 1. Scanning electron micrograph of rainbow trout spermatozoon that shows the head (H) and attached flagellum which is composed of a long principal piece (P) and a short end piece (E). Cytoplasmic vesicles (V) appear commonly in the anterior region of the flagellum. 3,000 \times . (Index line = 3 μ m.)

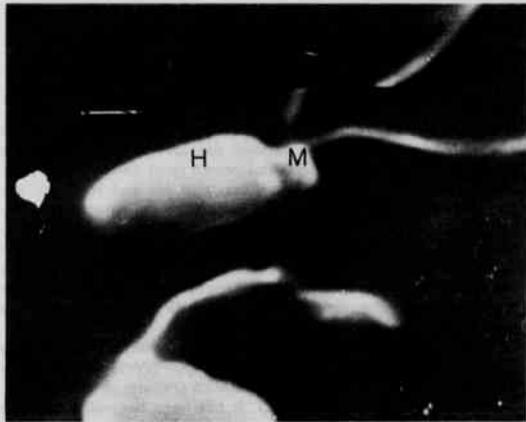


Figure 2. Scanning electron micrograph of rainbow trout spermatozoon head (H) lying at an angle that encourages identification of the mitochondrial collar (M). 10,000 \times . (Index line = 1 μ m.)

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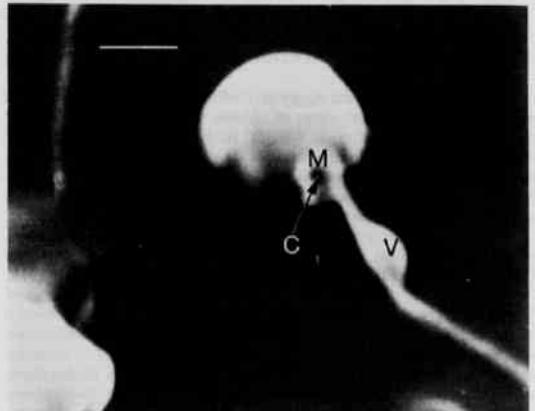


Figure 3. This scanning electron micrograph illustrates the cytoplasmic canal (C) that penetrates the mitochondrial collar (M). A cytoplasmic vesicle (V) is attached to the flagellum. 12,000 \times . (Index line = 1 μ m.)



Figure 4. This transmission electron micrograph demonstrates the relationship between the flagellum (F), cytoplasmic canal (C), and the mitochondrial complement (*) of the collar. The sectioned head (H) reveals the chromatin arranged in blocks that are composed of fibrillar material. 20,000 \times . (Index line = 1 μ m.)

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