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Note

Ultrastructure of egg membrane of rohu *(Labeo rohita)*

A. GOPALAKRISHNAN*, A.G. PONNIAH AND KULDEEP K. LAL
National Bureau of Fish Genetic Resources, Telibagh, Lucknow - 226 002, India
**NBFGR Cochin Unit, CMFRI Campus, Cochin - 682 014*

ABSTRACT

The fine structure of the egg envelope and micropyle of unfertilised spawned eggs of rohu (*Labeo rohita*) was observed using scanning electron microscope (SEM). The outer surface showed regularly arranged pores (dia. 0.20 - 0.25 µm) but was devoid of any filaments, fibrils or wrinkles. The micropyle was funnel shaped which exibited an outer pit (10 µm dia.) narrowing into a distinct canal (4.5 µm dia.). The larger diameter of the micropylar canal in comparison with the sperm head size of rohu and some other cyprinids make intergeneric and interspecific hybridisation easy in rohu.

The morphological features of egg envelops are highly adapted to the environmental conditions in which the embryo develops (Mikodina, 1987). The surface structure of fish egg envelope can also be a useful taxonomic criterion for identification of eggs (Guraya, 1986; Mikodina, 1987). Since the envelope of fish egg is relatively thick, the acrosomeless fish sperm gains access to the ooplasmic surface through "micropyle", a pore at the animal pole of the egg. The structure, size and development of micropyle greatly vary in different teleosts (Riehl, 1980; Guraya, 1986; Yamamoto and Kobayashi, 1992, Chen *et al.*, 1999). Wu *et al.* (1993) reported differences in surface and micropyle morphology between the eggs of normal (diploid) and triploid carp. Based on seven species-specific micropylar character suites, Chen *et al.* (1999) determined genetic interrelationship among four species of sparidae. As majority of the teleost sperm lack acrosome, the species-specific reaction between the sperm and the egg does not naturally occur during fertilisation. The size of the sperm head and the diameter of the micropylar canal prevents hybridisation between different genera and different species during mixed spawning (Jamieson, 1991). But among Indian major carps (catla, rohu, mrigal and calbasu), both intergeneric and interspecific fertile hybrids are commonly encountered (Mishra *et al.*, 1998) and morphological identification of some of the hybrids is often confusing. The present study was aimed at preparation of a base-line image of surface structure of all Indian major carps and their fertile reciprocal hybrids.

Ripe eggs were collected from female rohu spawners by hand-stripping, 6

hours after injection of 'Ovaprim' @ 0.4ml/kg body weight during peak breeding period. The eggs were fixed in ice-cold 2% glutaraldehyde, buffered to pH 7.3 with 0.1M cacodylate buffer over ice for 5 hrs. and osmified in 1% osmium tetroxide in 0.1M cacodylate buffer. The fixed eggs were dehydrated in ethanol series, critical point dried using CO_2 (Polaron E-3000) and sputter-coated with gold. The observations were made using philips SEM 501/B and photographed.

The unfertilised, ovulated rohu eggs (average diameter 750 µm) have a relatively smooth surface, characterised by regularly arranged round pores (Fig. 1) with diameter ranging from 0.20 - 0.25 µm and are distributed at a mean distance of 0.6 µm (range 0.39 - 0.86 µm).

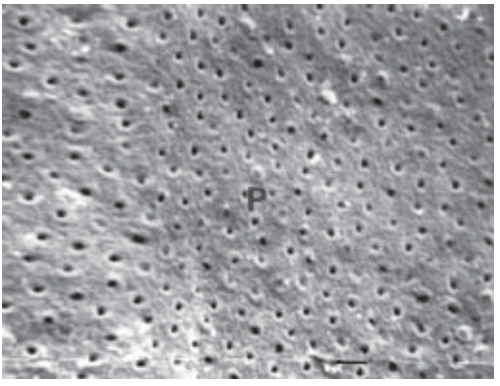

Fig. 1. SEM image of surface structure of unfertilized rohu egg (x 5000). P - pores Bars = 1 µm.

They have a simple morphology, in comparison to the complex outer covering of certain adhesive or pelagic eggs like *Clupea harengus* and *Cyprinus carpio*. The eggs envelope of the latter are studded with microfilaments, ornate surface structures or jelly coats (Guraya, 1986). The pores on the surface of ripe eggs represent the space earlier occupied by microvilli from the developing oocytes and granulosa cells (Guraya, 1986). With an outer pit (rim) diameter to 10 µm, the micropyle of rohu is funnel shaped (Fig. 2) leading into a canal having a diameter of 4.5 µm. The funnel shaped micropyle is characteristic of those species with sub-

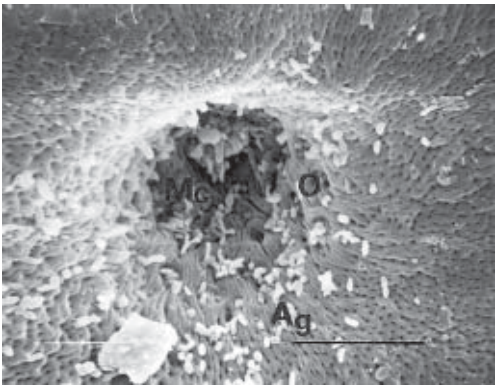

Fig. 2. SEM image of micropyle of rohu egg (x 2500). Ag - Agglutinates, Mc - Micropyle canal; O - Outer pit of micropyle. Bars = 10 µm.

merged eggs. Micropyle of pelagic eggs are only small depression of the surface envelope, while the same in demersal eggs are funnel-shaped with a wide outer pit and a canal (Mikodina, 1987). The diameter of the micropyle canal (4.5 µm) of rohu egg is larger than the size of the sperm head of both rohu (1.9 µm) and mrigal (2.2 µm) (Gopalakrishnan *et al.*, 2001), thus giving a clear scientific explanation for the free access of anacrosomal sperms (head diameter less than 4.5 µm) of other cyprinids into rohu eggs producing hybrids. The canal wall of rohu egg exhibited many folds or thickenings. Numerous agglutinates were also observed along the outer rim and canal of the micropyle (Fig. 2,3). As reported by Mikodina (1987) in other species, the agglutinates seen along the rim and micropylar canal of rohu eggs can be the residues of the cytoplasmic processes of granulosa cells. Mikodina and Makeeva (1980) demonstrated that inspite of a great similarity between the eggs of silver carp (*Hypophthalmichthys*

Fig. 3. Agglutinates (Ag) on the surface of rohu eggs. Bars = 10 µm.

molitrix), bighead (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*) and black amur (*Mylopharyngodon piceus*), the structure of their mircropyle and egg membranes differed greatly and were species-specific. Further study on eggs of other Indian major carps will reveal whether morphology of their micropyle and envelope differ from that of rohu.

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