EGG AND SPERM STRUCTURE OF THE BAGRID CATFISH, *Mystus nemurus*

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ABSTRACT

Egg and sperm structure of bagrid catfish, *Mystus nemurus* were observed using transmission (TEM) and scanning electron microscopy (SEM). The objective of the present was to evaluate structure of the egg and spermatozoa of bagrid catfish. The egg samples were preserved in a 10% formaldehyde and 1% gluteraldehyde fixative and dehydrated by a series of ethanol. Then after, the egg samples were soaked in xylene and wax then embedded and sliced by a microtome, stained and observed by a light microscope. Sperm samples were fixed in 2.5% McDowell-trump fixative and post fixed in cacodylate buffer osmium tetroxide. For SEM the samples were attached to cover slip previously coated with 0.1% poly-L-lysin and dehydrated by a series of acetone then critical point dried and coated with gold. While, for TEM the pellet samples obtained by gentle centrifugation were dehydrated in acetone and embedded in resin. Thin sections of samples were picked up on copper grids, doubly stained with uranyl acetate and lead citrate. The results showed that normal spermatozoa of bagrid catfish consist of three distinct parts; head, midpieces-sleeve, and uniflagellate of flagellum with nine peripheral doublets and single pair of central microtubulus ("9+2" patterns) of axoneme. The egg is oval and divided into three layers and can be classified as a non buoyant. Many micropores and single microphyle were detected on surface of the egg.

Key words: egg and sperm structure, *Mystus nemurus*.

INTRODUCTION

Spermatozoa and egg structure studies have been intensively examined. Information on spermatozoa ultra structure is needed to understand spermatozoa and egg biology, which is invaluable in developing spermatozoa cryopreservation methods (Lin *et al.*, 1996) and further enhancing artificial induce breeding efforts. Bagrid catfish is a popular catfish species currently being produced regionally in both semi-intensive and intensive culture system, and it is rapidly growing to be an important commercial species for Inland fisheries in Malaysia, Thailand and Indonesia. Reproductive biology of bagrid catfish has reported by Khan *et al.*, (1990). However, information on spermatozoa and egg structure of bagrid catfish has not been described. The objective of the present study is to describe the sperm morphology of bagrid catfish using transmission and scanning electron micrograph imaging and histological analysis.

MATERIALS AND METHODS

Sperm samples were fixed in 2.5% McDowell-trump fixative and kept for 48 hours. After 48 hours, sample was washed in 0.1phosphate buffer for one hour, and then samples were post fixed for one hour in cacodylate buffer osmium tetroxide and rinsed several times in the buffer. For scanning electron microscopy (SEM), samples were attached to cover slip previously coated with 0.1% poly-L-lysine. After dehydration through an ascending series of acetone, the samples were critical point dried in critical point dryer, and coated with gold in sputter coated and viewed by using electron microscope. While, for transmission electron microscopy (TEM), the sperm pellet obtained by gentle centrifugation were dehydrated in acetone and embedded in resin. Thin sections of samples were picked up on copper grids, doubly stained with uranyl acetate and lead citrate and examined using electron microscope.

Ovary samples were preserved in a 10% formaldehyde and 1% gluteraldehyde fixative for 48 h and dehydrated by a ethanol series of 50, 70, 80, 95, and 100% for, 1.5, 2, 2, 2 and 15 h respectively. After 15 h, samples were soaked in xylene and WAX, then sample were embedded and sliced by a microtome, stained and observed by light microscope.

RESULTS

Spermatozoa structure

Morphologically normal spermatozoa of bagrid catfish consist of three distinct parts; head, midpieces-sleeve, and flagella (Figure 1.A). The head

of bagrid catfish spermatozoa is rounded, sized 1.99 μm in length and 1.73μm in width. Their have a nucleus sized 1.44 μm in length and 1.24 μm in width, and its represent the chromatin. The head is completed with at least two-electron nuclear lacuna sized 0.23 μm, which penetrate in the nucleus (Figure 1.B).

The head is covered by cytoplasmic membrane and the nucleus is bordered by nuclear envelope (ne), and cytoplasmic channel (cl) is located between the flagellum (fl) and cytoplasmic membrane (cm), while the mitochondrial (m) are located in the midpieces-sleeve. Transmission electron micrograph imaging showed that a single spherical mitochondrial was appeared in sized 0.36μm (Figure 1C). The midpieces-sleeve is sized around 1.49 μm in length and 0.98 μm in width. The nucleus is connected to a short midpiece-sleeve and the flagellum by centriol complex, and the centriol complex was formed by distal centriol and proximal centriol, although its were not appeared in this observation.

Bagrid catfish spermatozoa are uniflagellate, the Flagellum sized around 4.10 μm in length and 0.19 μm in width. Cross section of the flagellar show the axoneme performed by nine peripheral doublets and single pair of central microtubulus ("9+2" patterns) (Figure 1.C). The flagellum membrane has one lateral projection (Figure 1.D).

Egg Structure

Preliminary observations reveal that the egg structure of bagrid catfish is oval and sized 1.41-1.48 mm in diameter and 0.34-0.50 mg in weight (Figure

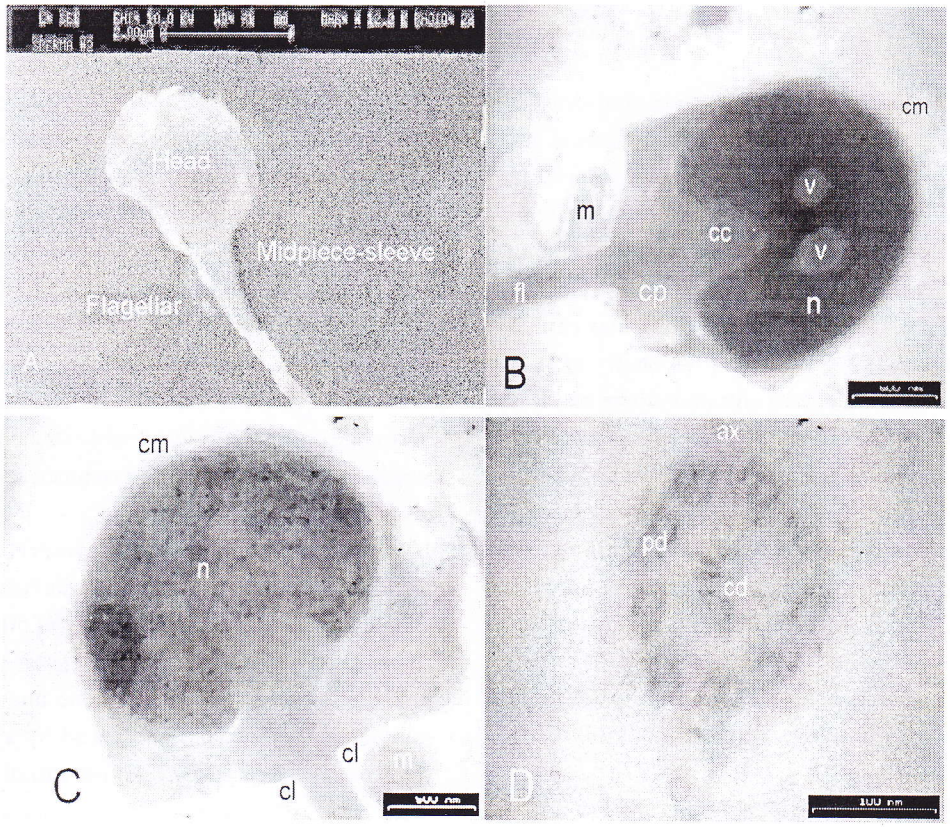

Figure 1. A. SEM imaging of bagrid catfish spermatozoa; B.TEM of bagrid catfish sperm showed two nuclear lacunas at the nucleus; C. The head is covered by cytoplasmic membrane and one mitochondria was presentthe at midpiece-sleeve; D. "9+2" patterns of axoneme and one lateral projections of the flagellum. Nucleus, n; flagellum, fl; nuclear lacuna, v; cytoplasm, cp; mithocondria, m; axoneme, ax; cytoplasmic canal, cl.

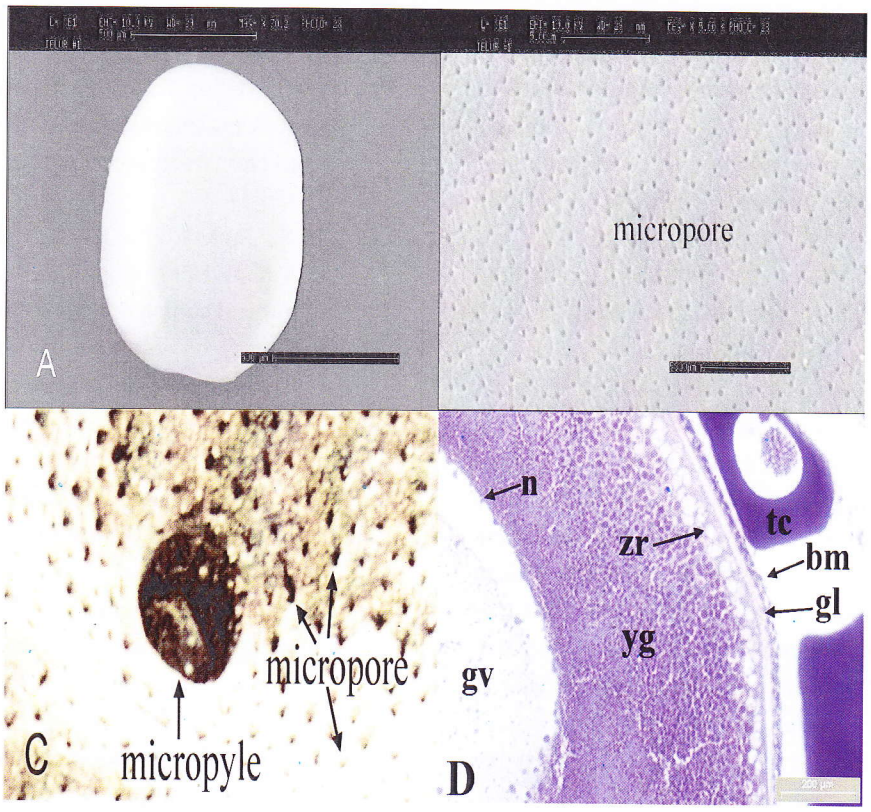

Plate 2. Structure of egg and ovarian follicle of bagrid catfish. (A). outside appearance of normal egg, (B) egg surface showing micro pores, (C). micropyle at higher magnification show microphyle canal (C) performance of egg clutch. (D) cross section of. egg show their layers (D) primary oocyte. tc, theca cell; bm, basal membrane; gl, granulose layer; zr, zona radiata; yg, yolk granulose; n, nucleus; gv, germinal vesicle.

2.A), and is divided into three layers, i.e. basal membrane, granular layer, and radiata zone, the yolk granulose, which is present under radiata zone and the nucleus consisting of a germinal vesicle (Figure 2.D.). The egg surface is smooth and contains high density of micro pores (Figure 2.B) one micropyle (Figure 2.C), and without an attachment apparatus.

DISCUSSIONS

In teleosts, the morphology of the spermatozoa is simple; the head is round or ovoid-shape, the middle piece is reduced to a mitochondrial ring and the flagellum present a typical "9+2" structure (Suquet *et al.*, 1993). The bagrid catfish spermatozoa are a typical aquasperm with a spherical nucleus, short midpiece, and short cytoplasmic collar. Head spermatozoa of bagrid catfish is rounded, and with no acrosome structure at the head. Acrosome structure has also absent in some species, for example in tilapia spermatozoa (Don and Avtalion, 1993), and turbot (Suquet *et al.*, 1993).

The acrosome function is to breakdown the micropyle pore for penetration and fertilization of the egg. Acrosome is not necessary for fish spermatozoa due to the fish egg have a micropyle pore which is always opening to permit sperm passage to fertilize the egg (Amanze and Iyvegar, 1990). Once fertilization occurs, the micropyle pore is barred by filamentous network (Bern and Avtalion, 1990). The midpiece-sleeve of bagrid catfish spermatozoa is short with single mitochondrial, a similar performance was found in turbot spermatozoa (Suquet *et al.*, 1993). Whereas a long midpiece-sleeve with at least nine mithochondrial was found in ocean pout (Yao, *et al.*, 1995).

Based on some literature review, there are two types of flagellum; uniflagellate and biflagellate.

The flagellum of bagrid catfish spermatozoa is uniflagellate with exoneme formed by nine peripheral doublets and single pair of central microtubules (9+2 structure), similar patterns were found in *Thunnus thynnus* and *Euthynus alletteratus* (Abascal *et al.*, 2002) and *Diplodus puntazzo* (Taddei *et al.*, 2001). Uniflagelate with "9+1" patterns of axoneme was found in tilapia (Bern and Avtalion, 1990), and biflagellate with "9+2" structure of axoneme is found in ocean pout (Yao, *et al.*, 1995).

The egg of bagrid catfish can describe as non-buoyant without attachment organ. Examples of other fish that do not have an attachment apparatus organ are *Sciaenops ocellatus,* and *Mugil cephalus* egg (Li *et al.*, 2000). The purpose of the attachment apparatus organ or adhesive filaments is to attach to the substrates when the eggs are released into water and protect them from being washed away in the water. Fish with an attachment apparatus organ include in *Lepadogaster lepadogaster, Diplecogaster bimaculatus* (Breining and Britz, 2000), Polypterus ornatipinnis (Bartsch, and Britz, 1997).

REFERENCES

Abascal, F.J., Medina, A., Megina, C., Calzada, A. 2002. Ultrastructure of *Thunnus thynnus* and *Euthynnus alletteratus* spermatozoa. *Fish Biology*, 60:147-153.

Amanze, D and Iyvengar, A. 1990. The micropyle: a sperm guidance system in teleost fertilization. *Development*, 109: 495-500.

Bartsch, P., and Britz, R. 1997. A single micropyle in the eggs of the most basal living actinopterygian fish, *Polypterus* (Actinopterygii, Polypteriformes). *Zoological Society of London*, 241:589-592.

Bern, O., and Avtalion, R.R. 1990. Some morphological aspect of fertilization in tilapias. *Fish Biology*, 36: 375-381.

Breining, T., and Britz, R. 2000. Egg surface structure of three clingfish species, using scanning electron microscopy. *Fish Biology*, 56: 11229-1137.

Don, J., and Avtalion ,R.R. 1993. Ultraviolet irradiation of tilapia spermatozoa and the Hertwig effect: electron microscopic analysis. *Fish Biology*, 42:1-14.

Khan, M.S., Ambak, M.A., Ang, K.J., and Mohsin, A.K.M.1990. Reproductive biology of a tropical catfish *Mystus nemurus* (C & V) in Chenderoh resorvoir, Malaysia. *Journal of Aquaculture Fish. Manage*, 2: 173-179.

Lin, F.L., Liu, L., and Dabrowsky. 1996. Characteristics of muskelluge spermatozoa I: ultrastructure of spermatozoa and biochemical composition of semen. *Trans. Am. Fish. Soc.*, 125: 187-194.

Li, Y.H., Wu, C.C., and Yang, J.S. 2000. Comparative ultrastructure studies of the zona radiata of marine fish eggs in three genera in Perciformes. *Fish Biology*, 56: 615-621.

Mattei, X. 1991. Spermatozoon ultrastructure and its systematic implication in fishes. Canadian *Journal of Zoology*, 69:151-158.

Suquet, M., Dorange, G., Omnes. M.H., Normant, Y., Le Roux, A., and Fauvel, C. 1993. Composition of the seminal fluid and ultrastructure of the spermatozoon of turbot (*Scophthalmus maximus*). *Fish Biology*, 42: 509-516.

Taddei, A.R., Barbato, F., Abelli, L., Canese, S., Moretti, F., Rana, K.J., Fausto, A.M., and Mazzini, M. 2001. Is cryopreservation a homogenous process? Ultrastructure and motility of untreated, prefreezing, and postthawed spermatozoa of *Diplodus puntazzo* (Cetti). *Cryobiology*, 42:244-255.

Yao, Z., Emerson, C.J., and Crim, L.W. 1995. Ultrastructure of the spermatozoa and egg of the ocean pout (*Macrozoarces americanus* L) an internally fertilizing marine fish. *Mol. Reprod. Dev.* 42, 58-64.