

# Electrophoretic Pattern of Serum and Yolk Protein With Relation to In Vivo Oocyte Development by HCG Hormone Treatment in Catfish(*Silurus asotus*)

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## INTRODUCTION

The egg development of various fishes is studied by various methods including electron microscopy(Matsuyama et al, 1991) and hormone treatment(Schoonbee et al. 1980). In addition to attempt to induce final maturation and spawning, the present experiments were conducted to investigate the influence of hCG upon reproductive cycles in catfish(*Silurus asotus*) with developing gonads and to stimulate the reproductive system during the rapid phase of gonadal development.

## MATERIALS AND METHODS

### Animals, hormone injections and blood collection

Catfish weighing from 100 to 300g reared in a freshwater pond( $26 \pm 1^\circ\text{C}$ ). The fish were anaesthetized with MS 222(1/10,000) and were injected intraperitoneally with hCG or 0.8% saline. Blood samples of fish before and after injection were taken from the caudal vein into heparinized vials. The plasma was obtained by centrifugation the blood.

### Preparation of SDS extracts and electrophoresis

Homogenation of fish eggs carried out in centrifuge tubes by homogenizer following 3-fold dilution using 2% w/v aqueous SDS. Each homogenate was centrifugated at 11,600g for 20 min after incubation at  $60^\circ\text{C}$  for 30 min as described by Scobbie and Mackie(1990) The extracts were analyzed by SDS-PAGE on 7.5% separating gel and 4.4% stacking g

### Electron microscopy

For TEM observations, the living specimens were fixed in 2.5% glutaraldehyde buffered with 0.1 M PBS, postfixied in 2% osmium tetroxide, dehydrated by graded series of ethanol, and embedded in Epon 812. Ultrathin sections were obtained from the same block by ultramicrotome with a diamond knife. The sections were double-stained with aqueous 5% uranyl acetate and lead citrate solution, and examined in a transmission electron microscope.

## RESULTS and SUMMARY

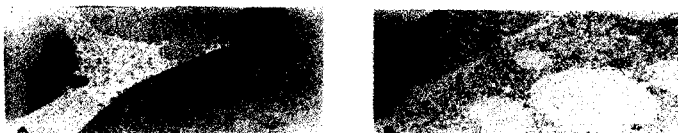
### Electrophoretic patterns of plasma and egg extracts

Figure 1 shows the electrophoretic patterns obtained when the SDS extracts of egg were subjected to electrophoresis. The results obtained show very close correspondence in the profiles for all the eggs in the presence and absence of some of the major or minor zones. The vitellogenin of hormone-treated fish stained more intensively than that of

sham-treated fish. However, the thickness of electrophoretic band in molecular weight for hCG-treated fish was slightly lower than that in saline control. These proteins showed some minor or main bands of plasma and egg extracts which migrated at positions corresponding to molecular weights of about 40,000 and 2120,000, respectively.



<Fig. 1>. SDS-PAGE patterns of plasma protein(A~D) and oocytes extracts(E~I) in female catfish, *Silurus asotus*. A, B, E, F:sham-treated fish. C, D, G, H, I:hormone-treated fish. M:marker protein(40~212kda).



<Figs. 2>. A. Many yolk granules are located in ooplasm of mature oocyte. B. A part of the distinct banding of zona radiata in mature oocyte. Scale bar represents 2 $\mu$ m.

#### Ultramicroscopic appearance of oocyte membrane

In contrast to the control fish, the ovaries in the catfish treated with hCG shows a considerable ultrastructural change under the electron microscope(Figs. 2). During fina maturation, the microvillar processes from the oocyte are seen no longer to penetrate deep into the extracellular spaces of the overlying granulosa cells. Prior to ovulation, the zona radiata becomes more compact, and devoid of pore canals, and there is a wide space between the vitelline membrane and zona radiata. We have further been able to initiate 100% oocyte maturation in follicles in vitro by treatment with hCG and a high temperature. It seems clear that a long acting preparation containing hCG can be successfully used in prespawning fish to advance the final events of gonadal maturation and initiate spawning.

#### REFERENCES

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