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# QUANTITATIVE STUDY OF VITELLOGENIN DURING THE PERIOD OF OOGENESIS IN A MATURED FEMALE FISH *Heteropneustes fossilis*,(BLOCH 1794)

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## ABSTRACT

*Heteropneustes*, is a genus of catfish native to Asia. *Heteropneustes fossilis* is a species, the only one in its family of air sac catfish found in India. It is found mainly in ponds, ditches, swamps, and marshes, but sometimes occurs in muddy rivers. It can tolerate slightly brackish water. It is omnivorous. It is a common air- breathing, venomous fish with its pectoral spines. This species breeds in confined waters during the monsoon months, but can breed in ponds, derelict ponds, and ditches when sufficient rain water accumulates. It is in great demand due to its therapeutic value. It is highly preferred in India and commonly known as *singhi*.

The present study dealt with the observations made on the secretion of vitellogenin during the maturation period, as well as its relational and fractional study in *Heteropneustes fossilis*.(Bloch 1794)

The study revealed that the level of vitellogenin is maximum in ovary and minimum in blood plasma during the period of oogenesis as vitellogenin is a yolk precursor protein . It is observed through the process of SDS-PAGE gel electrophoresis. Histological studies of the fish liver as well as fish ovary also revealed the presence of vitellogenin and vitellogenin follicles in respected tissues.

**KEY WORDS:** *Heteropneustes fossilis,* Vitellogenin, SDS-PAGE gel electrophoresis.

## INTRODUCTION

*Heteropneustes fossilis shown* in Fig.1 the air breathing catfish being the cheapest source of animal protein have drawn the attention of many fishery biologists relative to their cultural and management practices. It has a great demand because of its high nutritional and therapeutic value. Vitellogenin (Vtg) is a complex protein synthesized in the liver cells using oestrogenic stimulation. At the stage of the growth of oocytes, their accumulation takes place. After the synthesis of Vtg, it is transported to the ovary, where it gets incorporated into the growing oocytes through receptor mediated endocytosis and processed into smaller yolk proteins such as lipovitellin and phosvitin. These proteins are used as a nutrient source for embryos during various developmental stage. Aggarwal (2014) have studied circannual gonadal cycle in *H.fossilis*, and showed direct correlation with plasma levels of sex steroids. Aggarwal (2011) have discussed about seasonal variation in plasma vitellogenin. Ghose (2005) have worked on seasonal effect of melatonin on ovary and plasma gonadotropin in *H. fossilis*.

**Fig.1 Cat fish or *Heteropneustes fossilis***

In this study we dealt with the observation of vitellogenin in plasma and also its presence in liver and ovary. This may help in studying the maturation and oogenesis process of this commercially important cultivable fish.

## MATERIALS AND METHODS

### Fish

Female fishes used in the experiment are collected from the local fish market of Bhagalpur (Fig. 2). Then they were acclimatized in the laboratory for one week.

**Fig 2. School of *Heteropneustes fossilis***

### Plasma Protein purification

The purification of the protein from plasma were done with the method of gel eletrophoresis SDS-PAGE (0.1% SDS) as shown in Fig 3, according to Laemmli (1970), in discontinuous gels, including a 4.0% stacking gel and a 8% separating gel. Purified Vtg sample and protein molecular weight markers ( Genei ,India) were mixed with an equal volume of sample buffer ( 4% SDS, 8% , 2 – mercaptoethanol, 20% glycerol in 0.125 M Tris HCL , pH 6.8) and were heated in boiling water bath for 5 mins before loading. Then the bromophenol blue dye was added to it. The gel was run in tris – glycine – SDS ( 25mM tris base , 192 mM glycine , 0.1% SDS, pH 8.3) buffer at 200V for approximately 50 mins till the bromophenol blue dye reached the bollom of the gel. The protein components were stained with Commassie Brilliant Blue R250. The quantity of vitellogenin were observed with the analytical software of Densitometery.

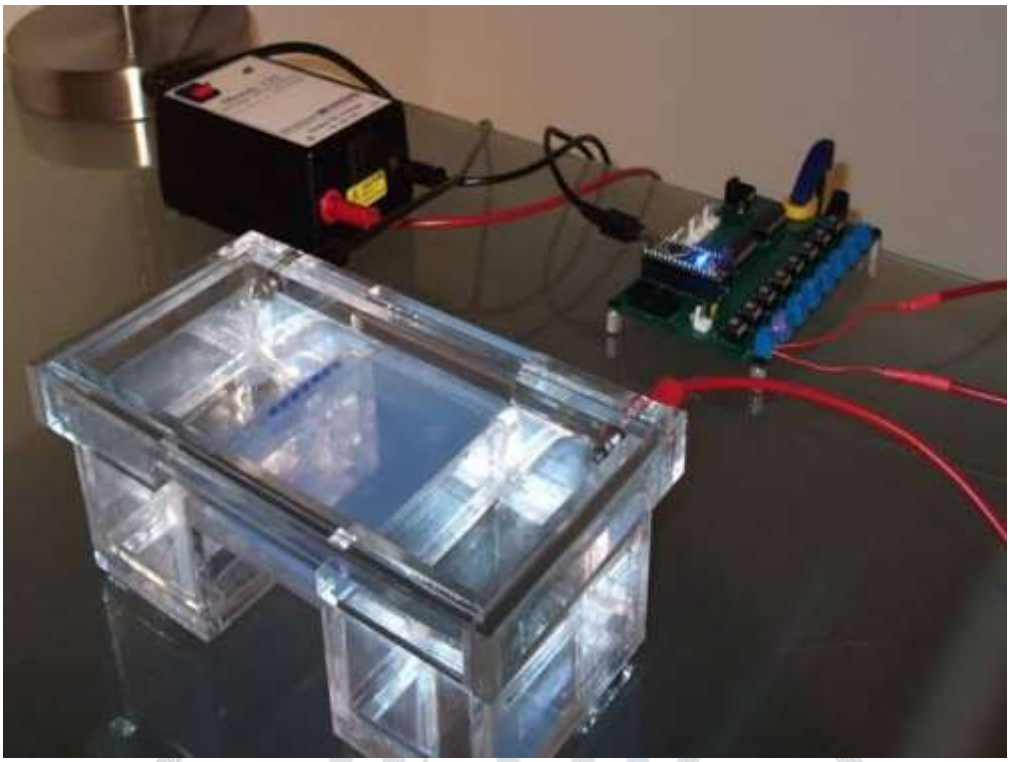

**Fig 3. Gel Electrophoresis set up for plasma protein purification**

**Histological study of liver and ovary**

After anaesthetization of the fish, the liver and ovaries were removed carefully and fragments of liver and ovaries of were fixed in aqueous Bouin's fluid. Subsequent to dehydration, the tissues were embedded in paraffin wax. All the tissues were serially sectioned at 5-6 μ m and stained with routine Haematoxylin and Eosin (H& E). This observation shows the presence vitellogenin in the section of liver and ovary in a matured female fish.

## RESULTS

Vitellogenin is the precursor of two yolk proteins , the highly acidic phosvitin (PV) phosvette in which about half of all amino acid residues are blocks of polyphosphoserin and lipovitellin, which is composed of a large and a small sub units (LV1 and LV2,respectively). The molecular weight of vitellogenin ranges from 114kDa to 600 kDa in a catfish (Mommsen and Walsh 1988). Several metabolic changes occur during vitellogenesis in the maturing female fish as reflected in the pronounced increase in liver weight, RNA content, lipid diposition , glycogen depletion, plasma protein, calcium and magnesium and phosphoprotein content (Wiegand M 1996 and Arukwe A 2003).

**Characterization of Vtg**

The molecular weight of *Heteropneustes fossilis* Vtg was estimated to be ~150 kDa using SDS-PAGE gel electrophoresis, and the purified Vtg revolved into a major band of ~150 kDAa and one minor band of ~130 kDa. The densitometery analysis showed that the quantity of vitellogenin is very high during oogenesis as compared to the other different stages in a female fish **(Fig 4)**.

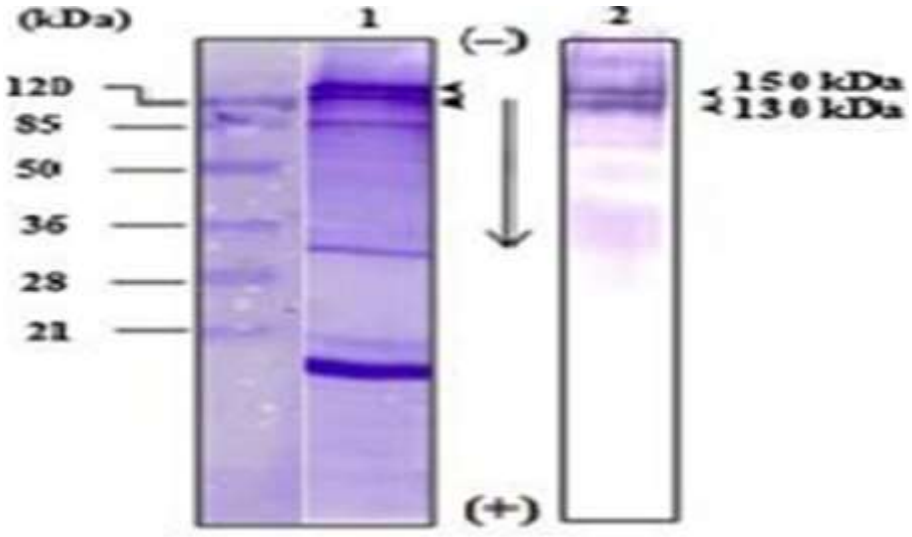


**Fig 4. Reducing SDS-PAGE of purified *Heteropneustes fossilis* Vtg run on 8% polyacylamide separating gel stained with Commassie Brilliant Blue. Lane 1. Molecular weight markers. Lane 2. Purified *Heteropneustes fossilis* Vtg.**

The amount of vitellogenin is estimated through Densitometery method, it was revealed that the isolated band has the amount of vitellogenin in different concentration asshown in Fig 5.

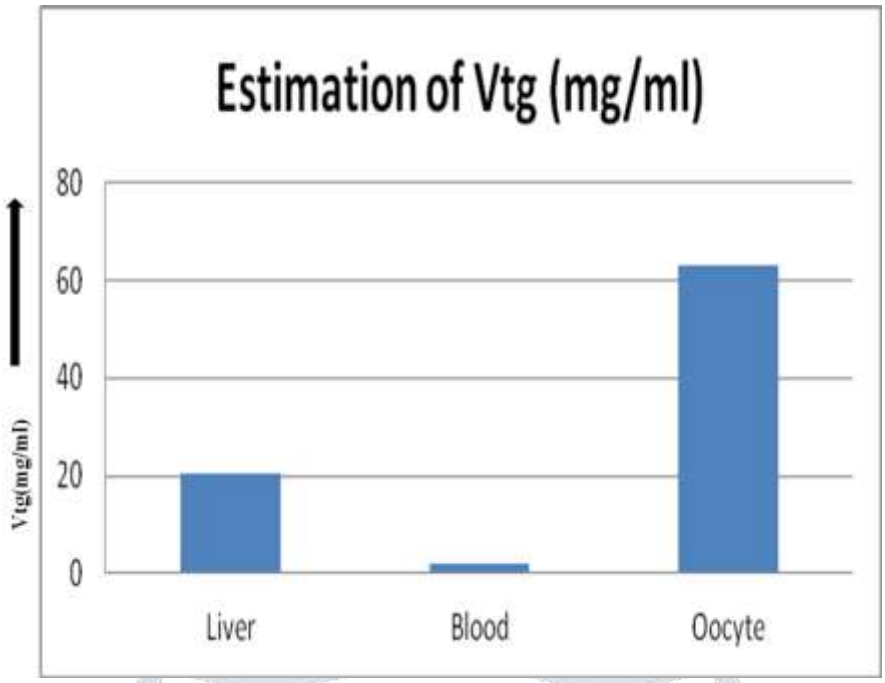


**Fig 5. Estimation of level of vitellogenin production in different tissues during oogenesis through Analytical Densitometry Software.**

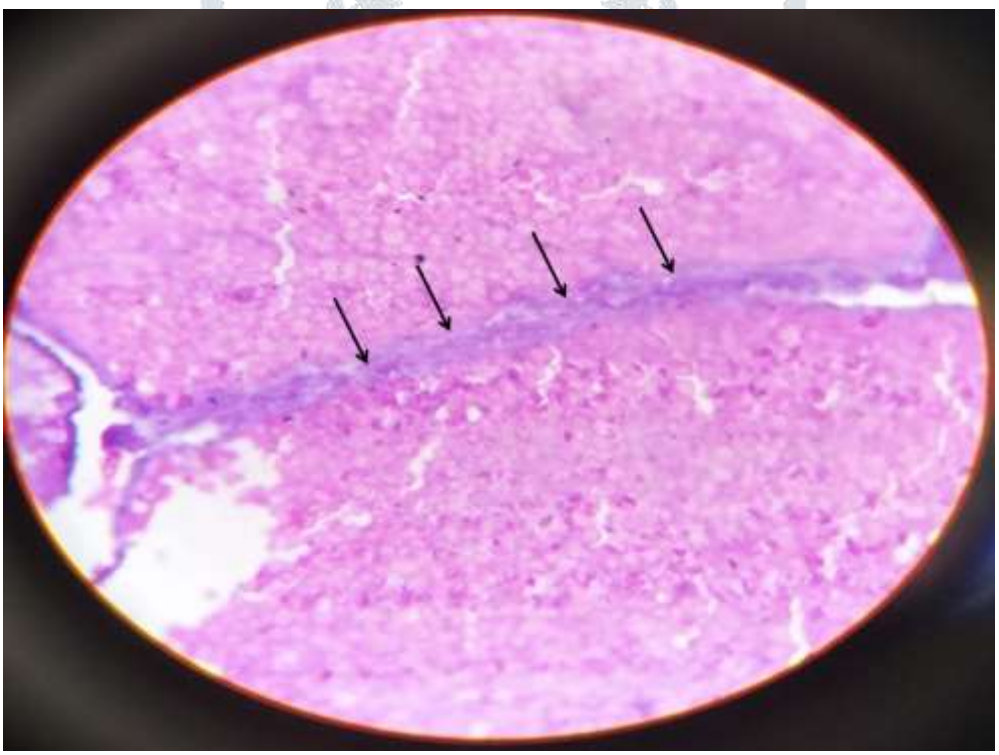

**Fig 6. Showing the presence of vitellogenin as bluish area in the hepatocyte of a female matured fish**

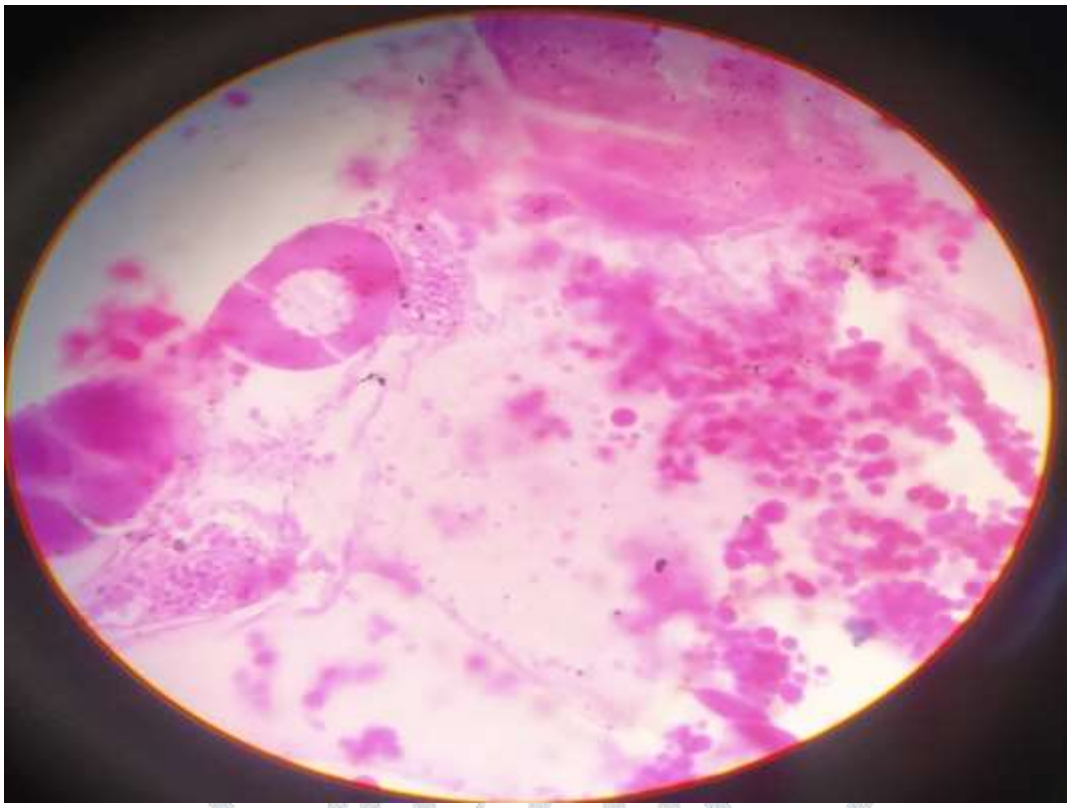

**Fig 7. Showing the presence of vitellogenic follicles in a ovary of matured female fish.**

Histological studies of the liver and ovary clearly shows the evidence of presence of vitellogenin in the liver tissue as shown in Fig 6 and the presence of vitellogenic follicles in the histological section of the ovary of the cat fish as depicted in the Fig 7.

## CONCLUSION

The secretion of vitelllogenin occurs in the liver of a matured female fish. It is again transported to the ovary during the process of oogenesis. Vitellogenin serves as different nutrient protein to the embryos during its various developmental stages. *Heteropneustes fossilis* has a great demand because of its high nutritional and therapeutic value. In the present piece of work, it was revealed that the molecular weight of the isolated vitellogenin through SDS-PAGE was around ~ 150 and 130 kDa and after densitometry the concentration of the total vitellogenin in different tissues were found as 20.56±0.51(Liver), 2.18±0.22(Blood) and 63.04 ±0.32(Oocytes) mg/ml respectively. This work is a comprehensive study of vitellogenin and its presence in liver and ovary that may help in studying the maturation and oogenesis process in a matured female *Heteropneustes fossilis.*

## DISCUSSION

In the present study it was observerd that during the process of oogenesis the synthesis of vitellogenin took place in the liver and diffuses in the blood plasma. The ovaries absorbed that vitellogenin from the blood and the yolk formation started. Because of this sequence of transportation , the quantity of Vtg differs in the blood , liver and ovary. Ovary contains maximum quantity of vitellogenin while blood contains mimimum quatity of vitellogenin during the process of oogenesis.

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