

STUDY OF THE ROLE OF MEMBRANOUS BLEBS IN THE NOURISHMENT OF OOCYTES IN *CHANNA PUNCTATUS* (BLOCH)

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ABSTRACT

Three types of yolk nourish the oocytes at their different developmental stages. It is the membranous blebs which facilitate such different types of yolky nourishment to the oocytes by carrying lipid droplets and other nucleolar inclusions till the maturity of the oocytes and store liporibonucleoproteins for their post fertilization embryogenesis.

Keywords: *Membranous Blebs, ex-vg Precursors, Ovarian Lipo-Depot*

INTRODUCTION

The proliferation of membranous blebs from the ovarian wall has been observed to begin at a stage when ovary was itself in the form of membranous sheet across the mid alimentary canal in a young fish normally during preparatory stage of oocyte in the month of September - October & January to February at the two occasions in the present study. These blebs are carried by numerous vesicles which in their turn are packed in a group of four to five large lobules surrounding a more or less oval lipid depot across the ovarian stroma.

Scharrer and Wuzzelmann (1969a); Riehl (1976a, 1978a) and Guraya (1986) have suggested the direct passage of nucleolar material from nucleus to ooplasm across the nuclear envelope in addition to that through nuclear pores. They referred to the blebs as the gateway for such direct passage, but they felt the need of more study over such transport. Hence, the present observation gives this account.

MATERIALS AND METHODS

Five specimens of female *Channa punctatus* were collected from the same pond preferably in the same week of each month throughout the year and sacrificed. Their ovaries were processed for the histological and biochemical detection. Morphological observations of ovaries were also done.

RESULTS AND DISCUSSION

At one occasion, in the month of September-October the originating ovaries are observed as yellowish-white membranes stretching over the mid alimentary tract and they grew to 'Resting stage' as 'pinkish fork shaped' morphologically during the month of January and February.

Till this stage no oocytes could be observed through naked eyes or under compound microscope. Only the membranous blebs could be observed, seem to be originating from their stratified stock located near the ovarian wall as observed under the compound microscope fitted with digital camera (five times magnification). In fact, the magnified view of these blebs suggested their origin from the tiniest droplets *denovo* originating centrally in the lumen of each transparent vesicle which later on becomes arranged along the periphery of a larger vesicle. Numerous such transparent larger vesicles are seen to be extruding from the ovarian wall. It has been observed that four to five lobules containing such transparent vesicles packed with membranous blebs were observed around a big mass of lipid (depot) (Fig.3a).



Figure 1: Ovary originating as a membranous sheet

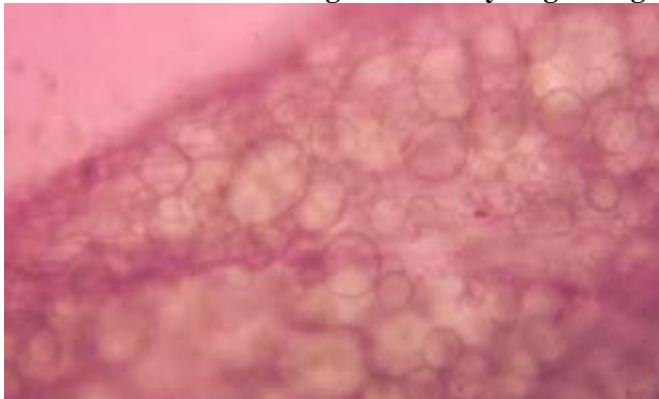


Figure 2a: The wall of the originating ovary showing proliferation of membranous blebs

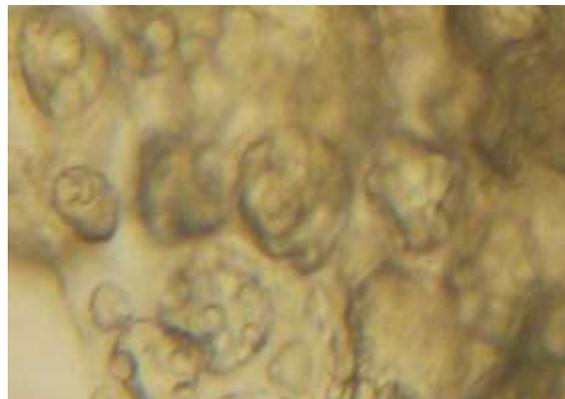


Figure 2b: T.S. of membranous ovary showing multi vesicular body (MVB) (x 600)



Figure 2c: Enlarged view of a MVB and its Liumen with numerous membranous micro blebs in (x 1000), (Alcian blue stain)



Figure 3a: Centrally placed yellow depot of lipid

PLATE 1



Figure 3b: Amidst lobules yellow depot of lipid

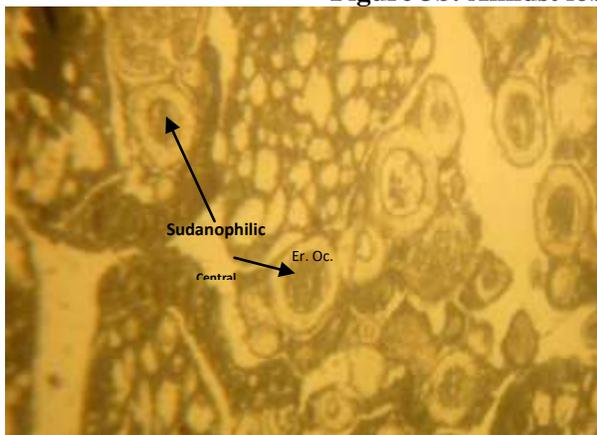


Figure 3c: (Sudan Black B positive early and mid vitellogenic oocytes (x 400))

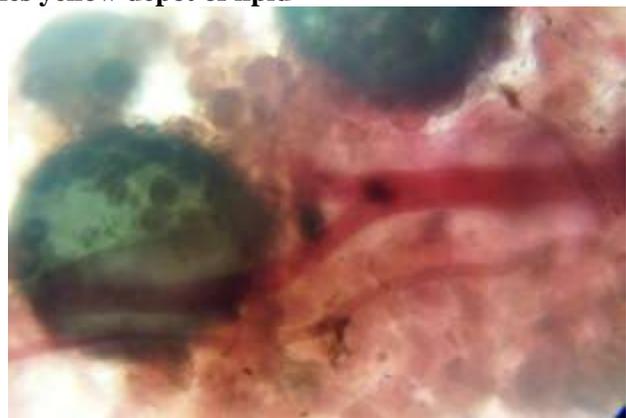


Figure 4: Membranous blebs anchored to the tip of stage-II oocytes



Figure 5: (T.S. through ovary with early and mid vitellogenic oocyte with granular cytoplasm (x 400) (H.E. stain) (GV = Germinal vesicle = nucleus))

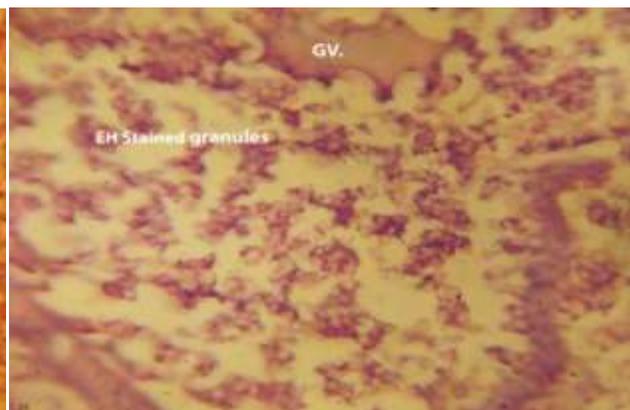


Figure 6: (A vitellogenic oocyte with broken germinal vesicle and scattered granules (x 1000) (H.E. stain))

PLATE 2

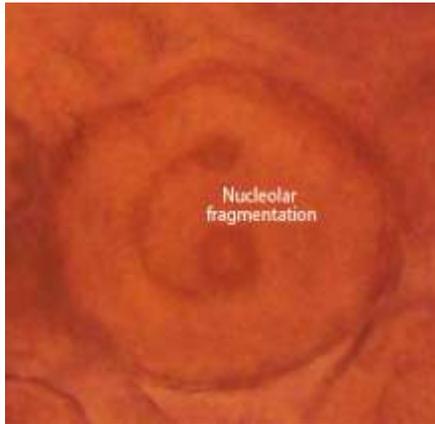


Figure 7a: Enlarged view of a chromatin nucleolar oocyte (x 400)

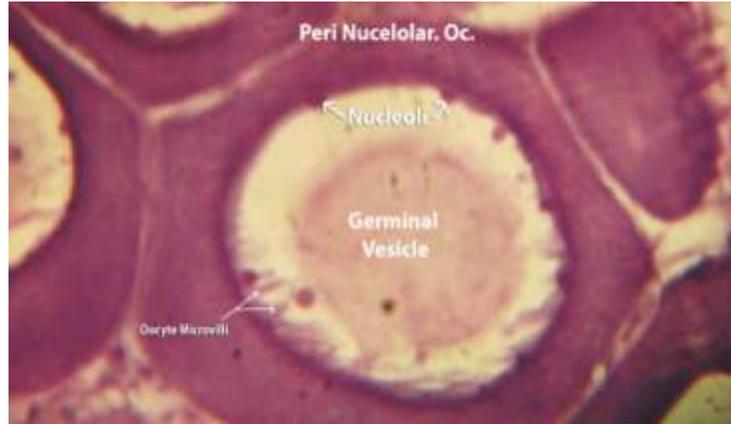


Figure 7b: T.S. of a Peri Nucleolar oocyte showing a number of nucleolar fragment along the periphery of germinal vesicle (x 600) (H.E. stain)



Figure 8a: T.S. of 'early maturing' ovary showing an enlarged view of peri-vesicular (early vitellogenic oocyte with corticle vesicle (x 400) (H.E. stain)

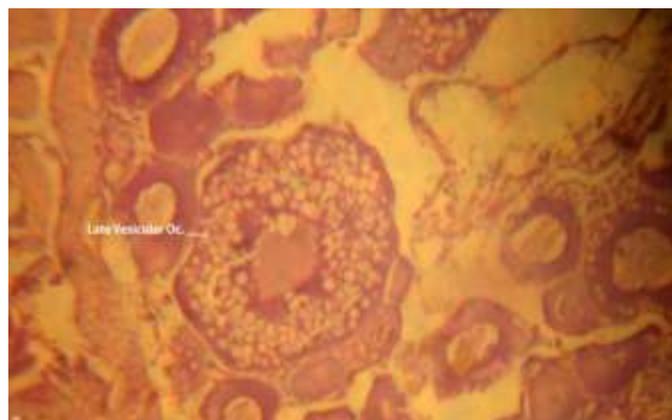


Fig. 8b T.S. through ovary with enlarged view of late vesicular oocyte (x 200) (H.E. stain)

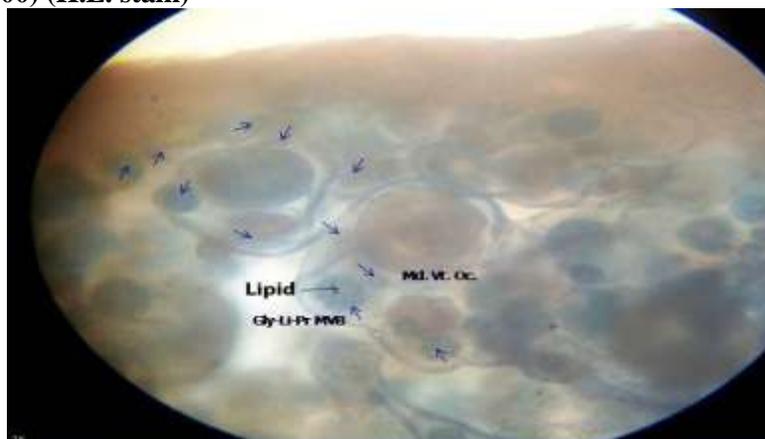


Figure 9: T.S. of maturing ovary with Alcian blue stained MVB carrying centrally located yellow lipid which sequenced towards vesicular oocytes (x 200)

PLATE - 3

Before delving deep into the results and discussions it is pertinent to mention here that since every month the catches of fish were having such fish which show their ovaries at different developmental stages, hence irrespective of the months, the ovaries were studied and arranged according to their developmental stages. Therefore, it was observed that an ovary originates as a membranous sheet (Fig. 1) when the proliferation of membranous blebs sets on (Fig. 2a). These blebs gathered at the periphery of a vesicle and numerous such vesicles gathered inside each lobule (Fig. 2b & 2c). Amidst four or five lobules centrally lies an oval, yellow lipid depot (Fig. 3b). The larger blebs carried sudanophilic lipid content to the centre (Fig. 3c) (Gopal Dutt and Govindan, 1967) and the glycoproteinous (Alcian blue stained) (Shahi *et al.*, 1979) granules at the periphery of each early vitellogenic oocytes (Verma *et al.*, 1981) indicating that each larger bleb has been impregnated with lipid-content carried from the yellow lipid depot placed centrally amidst the lobules across the ovarian stroma.

It has been observed that the membranous blebs lie anchored to the tip of blood channel along the late stage-II of the oocytes (Fig. 4). These blebs carrying two types of content- proteinous and lipid, have been observed gradually penetrating into the developing oocytes (early vitellogenic and mid vitellogenic oocytes) (Fig. 5). Later on at stage-IV (matured oocytes) the blebs have been found to be impregnated with lipo-ribo-nucleoproteins which later on scattered inside the whole ooplasm of the oocytes (Fig. 6).

In the present study the earlier views of R. patino and C.V. Sullivan (2002) regarding the extra-vg source of lipid acquisition by oocytes have been confirmed and it has been tried to view the basic process involved in it encompassing the role of membranous blebs. The tiniest blebs are generated inside the lumen of each vesicular structure which later on sequestered into the developing perinucleolar (Fig. 7a & 7b) and vesicular oocytes (Fig. 8a & 8b & 9) having fragmented nucleoli lying along the nuclear membrane and vesicles at their periphery. The purpose of blebs is to carry away the finer nucleolar extrusions into the ooplasm. The eosin-hematoxyline stained nucleolar extrusions along with the lipid filled blebs remain scattered inside the oocytes.

These nucleolo-ribosomal extrusions combine with the exogenous (hepatic) vitellogenin (vg) precursors (Nath *et al.*, 2007) and lipid (from indigenous depot) constituting lipo-ribo nucleo-proteinous yolk. These granules are packed inside the membranous blebs located inside the ooplasm at its periphery to be used up during further embryogenesis.

CONCLUSION

Thus, the present investigation confirms the role of membranous blebs which proliferate from the wall of originating ovary is to carry the nucleolar extrusions into the periphery of the perinucleolar oocytes and also carry the lipid miniscules from the ovarian lipo-depot to the synthesis of lipo-ribo nucleo-proteinous yolk with the aid of exogenous (hepatic) vitellogenin (vg) precursors.

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