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Structural changes of gonadotropic lobe in relation with gonads development of silver carp (*Hypophthalmichthys molitrix*) during maturation and ovulation

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Abstract. The paper has followed the evolution of gonatotropic lobe structure in relation with gonads development of silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844), female breeders taken from the Great Basin Pond 1 – Vaslui county, Carja Fish Farm. Ten specimens were analyzed after completion of vitelogenesis process, in the both maturation period (April-May) and ovulation period (June). On the histological sections of the ovary, during oocyte's maturation, secondary oocytes (stage VII^{A}), passing towards stage VII^{B} of maturation scale were distinguished as dominants while primary oocytes of young stages (III and IV^{A}) were rare. During ovulation a large number of mature oocytes (stage VII^{C}) are present. The pituitary histological sections showed an increasing number and size of the cells during maturation process while a progressive degranulation and vacuolation is observed in most gonadotropic cells during spawning.
Keywords: ovary, oocyte, hypophysis, maturation, ovulation.

Rezumat. În lucrare s-a urmărit evoluţia structurii lobului gonadotrop în raport cu dezvoltarea gonadei la reproducători femele de sânger, *Hypophthalmichthys molitrix* (Valenciennes, 1844), prelevate din bazinul Balta Mare din cadrul fermei piscicole Carja 1 – judeţul Vaslui. Au fost analizate 10 exemplare reproducători femele dupa încheierea vitelogenezei, în perioada de maturare (aprilie-mai) şi de ovulaţie (iunie). Pe secţiunile histologice de ovar din perioada de maturare se evidenţiaza dominanţa ovocitelor secundare (stadiul VII^{A}), care trec in stadiul VII^{B} pe scara de maturare şi rare ovocite primare din stadiile tinere (IV^{A} şi III), iar in perioada de ovulaţie se observă prezenţa ovocitelor mature (stadiul VII^{C}). Pe secţiunile histologice hipofizare, se observă creşterea numărului şi dimensiunii celulelor în perioada de maturare, iar în perioada de depunere a icrelor se observă degranularea progresivă şi vacuolizarea la majoritatea celulelor gonadotrope.
Cuvinte cheie: ovar, ovocite, hipofiză, maturare, ovulaţie.

Introduction. *H. molitrix* oogenesis includes, like in other teleosteen fishes, the following distinct periods: multiplication or oogonial period, growth (previtellogenesis, slow or protoplasmic growth and vitellogenesis, fast growth or trophoplasmatic growth) and maturation (Makeyeva 1992).

During the multiplication period occur successive mitotic divisions resulting in a pool of sexual cells (Takaski 1982). Polycyclic species, as the case of our specimens, from reserve of previtelogenetic oocytes will result the oocytes generation that develops into the final stages, the recruitment of the reserve stock of vitelogenic oocytes taking place continuously.

Ovarian fluid is a maternally derived liquid that surrounds the egg mass inside the female fish and is expelled during spawning (Taati et al 2010).

During the period of gametogenesis that the vertebrate fish undergo, the plasmic concentration of GtH-I grows, while the concentration of GtH-II remains at a low value. GtH-

I stimulates the gonads to produce 17β-estradiol for females and 11– ketosterone for males, as well as the hepatic vitellogenesis. GtH-II is responsible for the activation of the ovulation.

The concentration of GtH-II begins to increase before the sexual maturation and reaches maximum values during the spawning moment. It also stimulates the secretion of those gonadal steroids which induce the final maturation to the gametes (Kagawa et al 1998).

Some gonadal cells are situated in the dorsal part of the PPD (proximal pars distalis), among the ramifications of the neurohypophysis (NH) and are thought to produce GtH-I (FSH-follicle-stimulating hormone) and are situated on the ventral side free the hormone GtH-II (LH-luteinizing hormone) (Evans 2003).

Materials and Methods. For this histological research, there were taken oocyte and hipophysis samples from *H. molitrix* adult females aged between 5 and 6 years, with an average weight of 5-6 kg and originating from the Carja Fish Farm, Vaslui county.

The sampling periods coincided with the following:

- The maturation period, after the ending of the vitellogenesis (April-May 2008)
- The ovulation period which took place in June (10-25 June 2008).

The ova and hipophysis samples taken for the histological examination were modified according to classic methods. The fixation was made with Bouin and formol and, after inclusion in paraffin, the pieces have been segmented to 5 μm (hypophysis) and 7 μm (oocytes) with the help of microtome Minot.

The estimation of the oocyte development was made according maturation scale for the common carp females (Steopoe et al 1967) and Asiatic cyprinids (Nicolau & Steopoe 1970).

The size of oocytes was determined with the micrometer ocular Zeiss. There were obtained microphotographies with the Carl Zeiss microscope, Axio imager A1, photo camera Canon, 10 mega pixels, 4x optical zoom.

Results and Discussion

The evolution of the gonads. *The maturation period* is brought out in the samplings from April-May 2008, after the end of the vitellogenesis. Gradually, the oocytes pass to the stage $VIII^B$ on the evolution scale from a histological point of view (IV-V ichthyological scale) the nucleus migrates towards the cytoplasmatic sphere next to the micropile. The nucleoli are vacuolated and gathered towards the centre of the nucleus and the nucleus membrane disintegrates.

The oocytes stay in this stage 20-25 days. It is a favourable period for the hormonal induction.

On the histological section of the ovary, the oocytes are predominant, but there are also primary oocytes resulted from the earlier stages (IV^A şi III) (Figure 1).

The ovulation period. The ova sampling from 10-25 June 2008, histologically modified, showed the presence of mature oocytes with the vitellogenesis finished, before the expulsion out of the ovum.

From a histological point of view, we can notice the presence of oocytes in VII^B and VII^C stages, with a diameter of 1100-1200 μm (Figure 2).

The radiated zone is thick and covered with a jelly-like coating synthesized by follicular cells. This coat helps the surface cells to fix when they are laid in the water.

Inside the ovary, the number of the young oocytes is small and they undergo different stages of development.

When the oocyte is big, the vitelline content is divided and the hypertrophied follicular cells invade the ooplasm for phagocytosis (Takaski 1982).

The follicular atresia can be seen at the microscope under the form of a compact structure. After ovulation, the remaining oocytes continue their evolution representing the second or the third elimination.

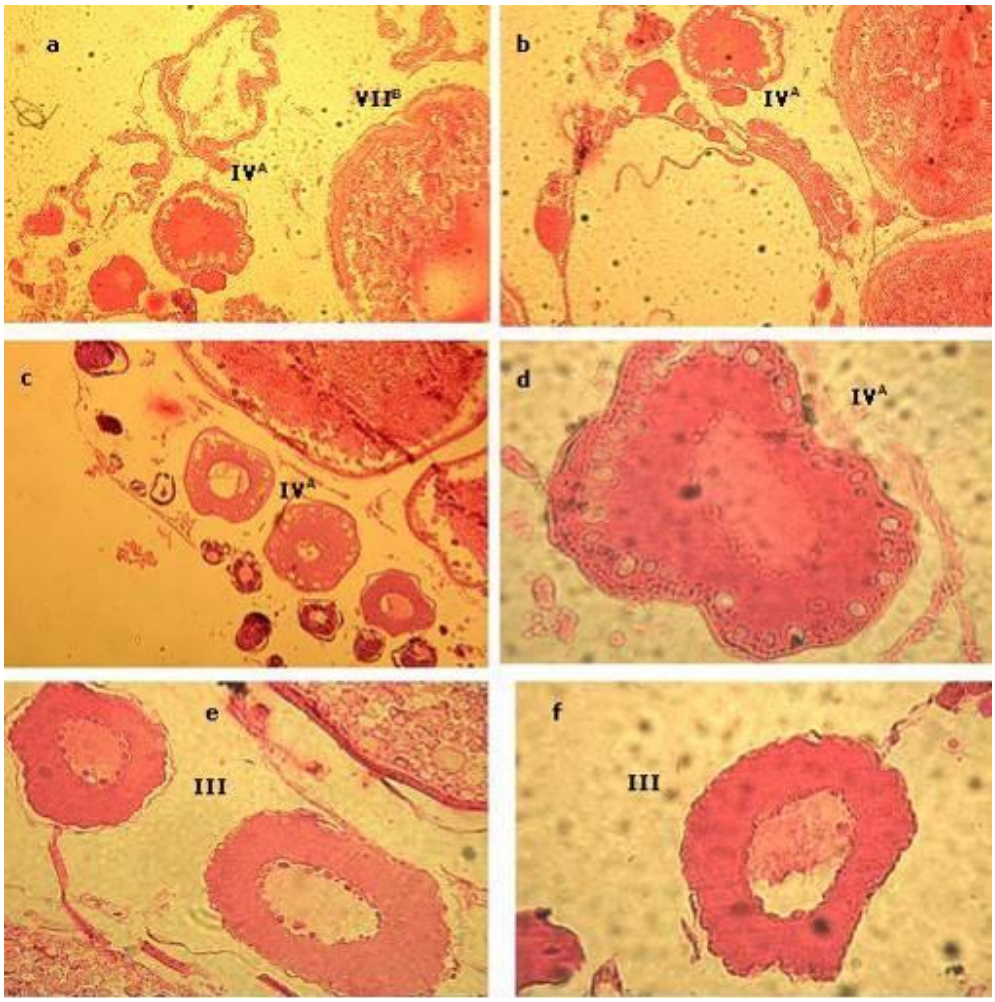

Figure 1. Oocytes during final maturation period (May 2008).
a- oocytes stage VII^{A}; b, c- oocytes stage VII^{B} (1100-1200 µm) with the nucleus towards micropile; d, e, f- oocytes stage VII^{B} – details. Col. HE, ob. 10 (a, b, c), ob. 40 (d, e, f).

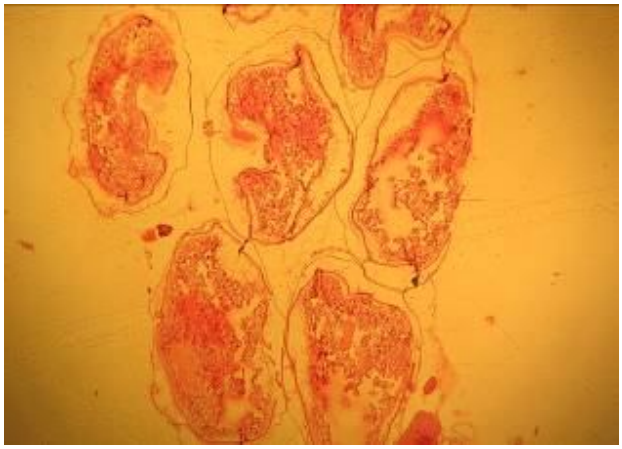

Figure 2. Oocytes in stage VII^{C}, atresia and ova elimination, ruptured follicles. Col. HE, ob.5.

The evolution of the gonadal lobe. *The maturation period.* For the maturation period, one can notice the display of the gonadal cells with a grainy texture near the capillary blood vessels where they pour their secretion product (see Figure 3). The gonadal area is characterized by the increase in number and the dimension of cells.

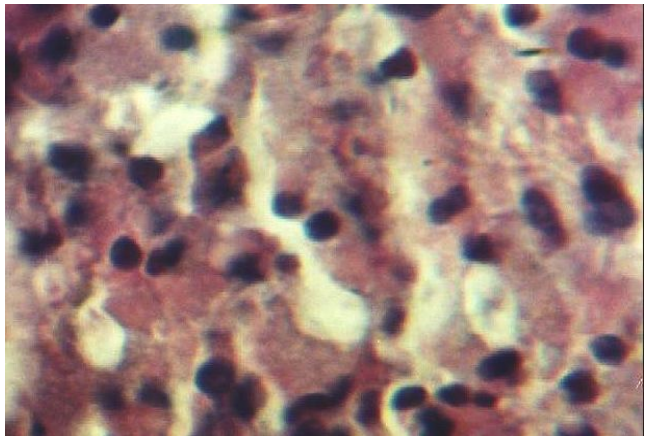

Figure 3. GTH cells (gonadotrope cells), displayed into strings, directing to the blood vessels, col. HE, ob.90.

After they have spawned, cells lose their polarity to the blood vessels.

The ovulation period. During the spawning period, the gonadal area considerably modifies. A progressive degranulation can be noticed, as well as an internal vacuolization, happening at almost all cells. In certain areas, cells are hypertrophic, they are no longer granulated and almost completely vacuolized (see Figs 4-6).

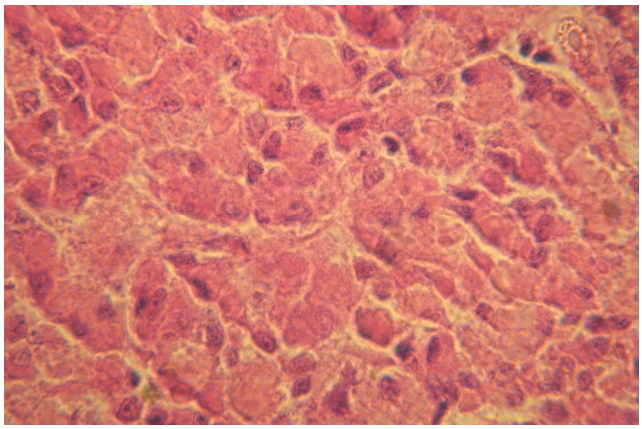

Figure 4. GTH cells partially degranulated, pyknotic nuclei, col. HE, ob.40.

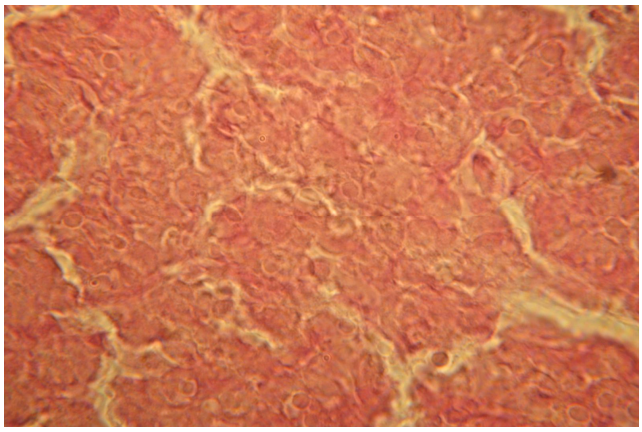

Figure 5. GHT hypertrophic and vacuolized cells during the spawning period, col. HE, ob.40.

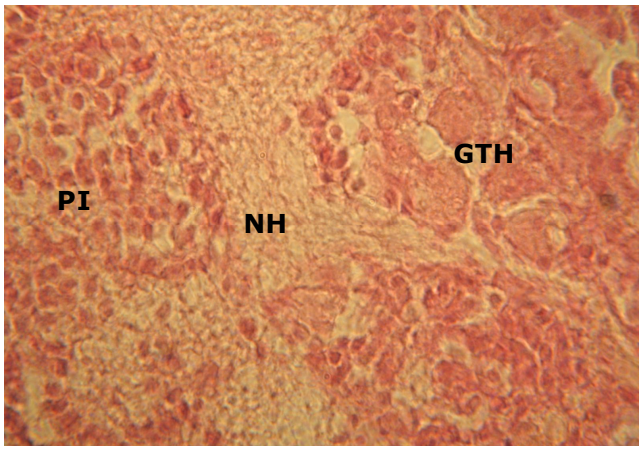

Figure 6. GHT hypertrophic and vacuolized cells, one can notice the neurohypophysis (NH) and the intermediary lobe (PI), col. HE, ob.40.

Conclusions. Following the histological analysis performed on the ovary of mature silver carp females, it results that, in the period when the research was conducted, the oocytes evolution was normal, in the condition established by the Carja Fish Farm, Vaslui.

The continual oogenesis, characteristic to polycyclic species, as the case of silver carp, is well represented on the histological sections. Thus, in the ovary there are the vitellogenetic oocytes which, during the maturation period, undergo structural modifications specific to this process; the process ends with the ovulation and the young remaining oocytes continue their evolution.

Starting from the comparison of the hypophysis structure during the second period of reproduction (maturation and ovulation), one can notice the presence of a correlation between the presence of secretion globules of gonadal hormones and the development stage of female gonads *H. molitrix*.

To conclude, we can state that, gonadal cells have a secretion activity cycle correlated with the oocyte ovulation, playing an important role in the case of vitellogenesis and oocyte's maturation.

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