

Induced breeding of *H. molitrix* and histological observations on the development stage of oocyte

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Abstract

The aim of the present study was to induced breeding of *H. molitrix* and to observe gonads maturation after the injections with the synthetic hormone Nerestin.

Males and females of *H. molitrix* were provided from the Cârja farm 1-from Vaslui county. All the breedings were injected with Nerestin 1, the females received two doses of 20%, respectively 80% of the total dose, within 24 hours, and the males received only the two doses representing 1/3 and 2/3 of the dose total administered to females.

For histological examination, oocyte samples were collected from adult *H. molitrix* females. The sampling periods overlapped with the maturation period after the vitellogenesis (April-May 2016) and ovulation, which took place in June 2016.

The ovulation process is determined by internal factors and assured by the normal development of the body; in the case of induced breeding is triggered by the administered gonadotrophic stimulants. Our results showed that in order to obtain good quality eggs a great importance must be given to the time of administration of gonadotrophin stimulants, and the quantity and quality of the introduced substance.

The biological material taken for the histological examination was processed by classical methods. Fixation was done in Bouin and Formalin, and after inclusion in paraffin, the pieces were cut at 7 μm by microtome SLEE. During maturation period, the oocytes gradually pass into stage VII^B, the nucleus migrates to the animal pole in the cytoplasmic area at the micropyle. During the ovulation period, the histological sections show the presence of oocytes matures with finished vitellogenesis, prior to expulsion from ovary. Histological, it was noted the evidence of the oocytes in stages VII^B and VII^C.

Oocytes remain at this stage for 20-25 days. It is the optimal period of hormonal induction until the beating conditions are formed

Keywords: Nerestin, maturation, ovulation, oocytes

1. Introduction

The reproductive cycle must ensure a sufficient quantity of mature egg cells, which is possible only within the regular process of the oogenesis [4].

In cyprinids, introduced three stages of oogenesis [3]:

- the synaptic path (from oogonia to oocytes in diplotene) - multiplication;

- growth (previtellogenesis, slow growth or protoplasmic and vitellogenesis, fast growth or trophoplasmatic);
- and rapid growth including maturation of the oocytes.

During the multiplication period, successive mitotic divisions result in a sexual cell reserve. In polycyclical species, as in the case of our fishes, from the previtellogenetic oocyte reserve will result the generation of oocytes that evolve to the final

stages, recruitment of vitelogenetic oocytes from the reserve stock takes place continuously.

2. Materials and Methods

In order to verify the effectiveness of the injections with neristin, two experimental lots of 15 specimens (1:1) were used, which were measured and weighed.

To stimulate maturation and ovulation of breeders under 10 kg, we used Nerestin 1, following the reproduction indicators, such as, maturation percentage and fertility percentage.

Administration of Nerestin was split, in two different doses of 20% and 80%, over a 24 hour interval, males receive only the second dose representing 1/3 - 2/3 of the total dose administered to the females. The total recommended dose for females in the 12-24 hours range is 0.3-08 ml / kg body weight at 21-26 ° C.

Two experimental lots of 30 fish (1: 1) were used to check the effectiveness of the injections with neristin, fish which were measured and weighed.

For histological research, oocytes were sampled from adult 4-5 year old *H. molitrix* females and an individual weight of 5-6 kg, from the Cârja 1 farm - Vaslui county.

The sampling periods matched with the maturation period, after the vitelogenesis (April-May) and the ovulation period, which took place in June.

The biological material for the histological examination was processed by classical methods. The fixation was done in Bouin and formol, and after inclusion in paraffin, the pieces were cut at 7 µm using the microtome SLEE. The sections were stained with hemalaun-eosin.

The assessment of the stages of oocyte development was made after the maturation scale developed by Steopoe I. (1962, 1967) [5,6] for Asian carp and Asian Cyprids.

The estimation of the size of the oocytes was done with the micrometer ocular Zeiss, and the photophotographs were obtained using the Carl Zeiss microscope.

3. Results and Discussions

After completion of vitelogenesis, as with all bone fish, the processes of the maturation division begin. Until the beating conditions are formed, the oocytes are in the resting state.

The formation of beating conditions induces hormonal stimulation to fish, and under their effect begins in the ovation of the maturation division process.

The period of oocyte development that lasts from the beginning of the division of the primary ovocyte to the metaphase of the second meiotic division is called final ovulatory maturation [2]. This is the primary condition for oocytes to be able to fertilize.

The process of ovulation induced by the internal factors ensured by the normal development of the organism is triggered, in the case of natural reproduction directed, by the gonadotrophic stimulants administered, experiments demonstrating that the obtaining of good quality eggs depends, apart from the time of administration gonadotrope stimulants, to a large extent, on the amount and quality of the stimulant substance introduced.

The lots of female and male hormone stimulated male breedings should be as homogeneous as possible to obtain good technological indices. In order to verify the homogeneity of breeding breeds used for breeding, the coefficient of variation was calculated for the studied character (weight), both in females and in male males, using the mean values and standard deviations presented in Table 1.

The analysis of the values calculated for the coefficient of variation (Cv) suggests that the lots used in the reproduction campaign are very homogeneous (Cv <10%) showing only a little variation in the body weight (table 2).

Breedings need to accumulate about 1300-1500 degrees days, calculated from 1 January to 1 June [1], in order to reach the stage of maturation of the gonads. Under climatic and temperature conditions at Cârja, these days are accumulated in early June.

Due to the fact that the stage of maturation of the gonads in the female *H. molitrix* reached stage VII^B on June 1, according to the scale proposed by Steopoe et al. (1967) [6], characteristic of stage VII^B is the peripheral location of the nucleus (in the animal pole) taking into account the considerations the accumulation of days grades was tested for breeding the first batch of *H. molitrix* on 07.06.2016.

After the first injection to the females at 7 am, the breeders were introduced separately by sex in the maturation basins, each of 15 fish / basin, and after the second injection made after 24 hours, the males

were first introduced into the circular basin, followed by the females.

After few hours after the second dose, the males started to follow the female at an intermediate level of the basin.

This behaviour has progressively increased, with males coming in contact with females. This behaviour precedes ovulation.

Table 1. Average values and standard deviations for weight and length in males and females used

Species	Lots used for reproduction	Lot 1		Lot 2	
		W (kg) (M±SD)	Lt (cm) (M±SD)	W (kg) (M±SD)	Lt (cm) (M±SD)
<i>H. molitrix</i>	femels	6,5±0,21	75,8±2,87	6±0,25	68,7±2,81
	males	5,9±0,21	68±2,83	5,4±0,31	62±2,93

Table 2. The coefficient of variation for weight for the breeding lots used in the breeding campaign

Species	Breeding lots	Coefficient of variation CV (%)	
		femeles	males
<i>H. molitrix</i>	Lex1N	4,838	5,280
	Lex2N	4,272	5,734

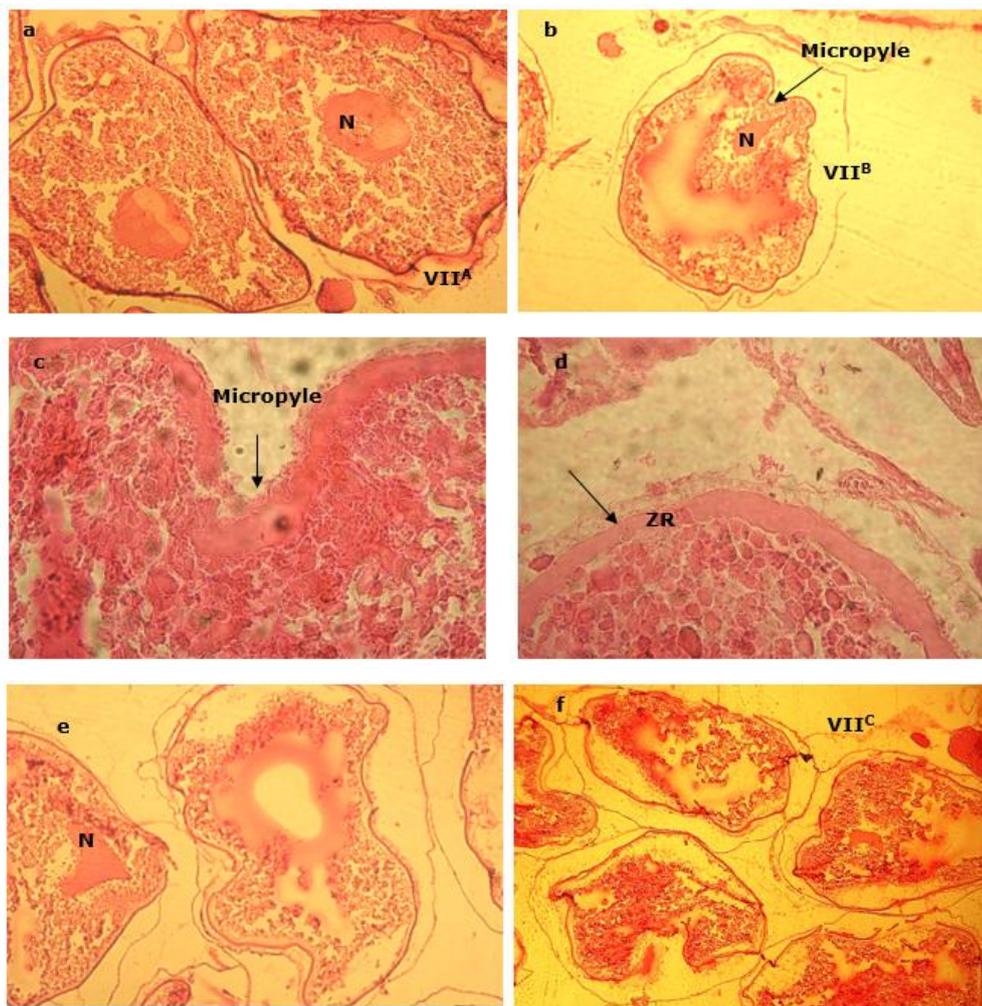


Figure 1. Oocytes during the final maturation and ovulation period
a- Oocytes stage VII^A; b, e- Oocytes stage VII^B (1100-1200 μm) with the nucleus migrated to the micropyle; c, d- ovulate stage VII^B – detail; f - Oocytes stadiul VII^C, atresia and ova elimination, ruptured follicles.
Col. HE, ob. 10 (a, b, e), ob. 40 (c, d), ob. 5 (f)

After a while the male and female climb to the surface, following each other, this behaviour generalizes quickly to the whole lot and repeats itself a few times, expelling eggs and sperm to be done simultaneously. Hydrated eggs appear in the circular box about 30-45 minutes after the expulsion of the first eggs.

The first index of the normal or abnormal character of development is the percentage of fertilization. It is determined at the stage of the microcellular morula, by analysing a number of 100 eggs in the magnifying glass or binocular, separately from the normal division and the abnormal divisions, and then the percentage of fecundation is calculated.

The percentage of 70-90%, in general, shows that the sexual products are of good quality and the embryonic development will normally take place (table 3).

Table 3. Situation of the main technological indices in the experimental work on the species *H. molitrix*

Technological indices analyzed	UM	Lot 1	Lot 2
Number of females injected	specimens	15	15
Average females weight	kg	6,5	6
Total female weight	kg	97,5	90
Number of mature females	specimens	13	12
Percentage of maturation	%	86,66	80
Percentage of fertilization	%	86	84

The advantages of gonadal stimulation with synthetic preparations analogous GnRH, are: they have a strong action and can have a low dose effect for stimulating LH [7]; there is a standard preparation so the results will be constant dependent only on the condition of the fish.

In the sections: The maturation period is highlighted in the samples from April to May 2016, after the finish of vitellogenesis. Gradually, the oocytes pass from stage VII^A (figure 1.a) to stage VII^B (figure 1.b) on the histological evolutionary scale (IV-V ichthyologic scale), the nucleus migrates to the animal pole in the cytoplasmic area at the micropyle (Figure 1.c) The nucleotides are vacuolized and grouped in the middle of the nucleus, and the nuclear membrane disintegrates.

The first maturation division is produced, which is a reduction division, the first polar globe is eliminated, and the sex cells become secondary oocytes (oocytes II).

Oocytes remain at this stage for 20-25 days. It is the optimal period of hormonal induction until the beating conditions are formed.

The ovulation period is highlighted in the samples from June 2016 showed the presence of mature oocytes with the vitellogenesis terminated, before to expulsion from the ovary.

Histologically, it noted the presence of oocytes in stages VII^B and VII^C which are reaching the diameter of 1100-1200 µm (Figure 1.e, f).

The process of the maturation division stops in the metaphase of the first maturation division, and in this state the ovum (VII^C stage) is removed from the follicle and ovulation respectively.

The radiated area is dense and covered by the gelatinous coating synthesized by follicular cells. This serves to fix the ovules on substrate when are inserted into the water.

The maturation divisions continue after the penetration of the spermatozoid when the second polar globe is removed. In the ovary, the number of non-immature oocytes is small and at various stages of development. There are also some relics of the ponte and the phenomenon of atresia.

When the oocyte is high, the vitelline content is fragmented, and the hypertrophied follicular cells invade the ooplasm for phagocytosis. Follicular atresia appears at microscopy in the form of a compact structure.

After ovulation, the remaining oocytes continue to develop, as the second, possibly the third ponte (Figure 2).

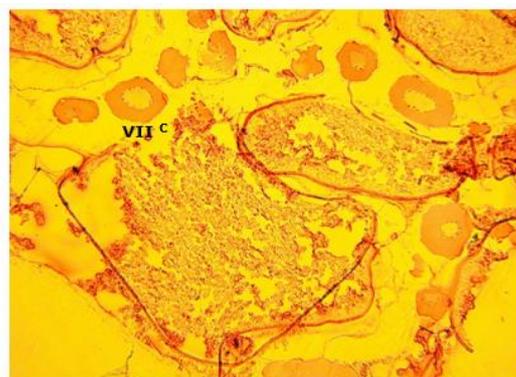


Figure 2. Ovarian section after ovulation Relic pontes (VII^C oocytes) and young remaining oocytes that continue their evolution. Col. HE, ob. 10 (foto original)

3. Conclusions

The use of the nenerstin, at stimulation of maturation and ovulation in *H. Molitrix*, has resulted in higher values of specific technological indicators. Norestine 1 given in two halves at 24 hours, at a water temperature of 21 °C, of which the first dose represents 20% of the total dose, is able to produce the maturation of the *H. molitrix* species. The females gave birth to suitable fertilizers.

From the histological analysis carried out by us on the ovary of the mature female females, showed that during the researched period the evolution of oocytes was carried out normally, under the conditions of the fishery Carja 1, Vaslui county. Oocytes gradually pass into histologically stage VII^B, the nucleus migrates to the animal pole in the cytoplasmic area at the micropile. In ovariectomic samples after administration of dioeza II, the presence of oocytes in stages VII^B and VII^C, oocytes mature with vitelogenesis terminated prior to expulsion from the ovary, is observed. After ovulation can be observed relives of pontus and terrestris, the remaining young oocytes continue its evolution.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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