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Structural Changes in the Ovary of *Heteropneustes fossilis* (Bloch) Exposed to Highest Magnesium Concentration during Various Stages of Reproductive Cycle

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ABSTRACT

The fish Heteropneustes fossilis (Bloch) belonging to experimental group were gradually adapted from 10 m mol l^{-1}, 20 m mol l^{-1}, 35 m mol l^{-1}, 55.0 m mol l^{-1} and 80 m mol l^{-1} (each step lasted for a day). Exposed highest[1] magnesium chloride ($MgCl_2.6H_2O$) solution the animal could not survive for more than 7 to 8 hours and is found lethal. Important cytological changes were observed during Various Stages in the ovary. Thick ovarian wall and ovigerous lamellae are ruptured in comparison to control group. But early chromatin nucleolus and late chromatin nucleolus stages are found highly basophilic than control during post-spawning period. During pre-spawning and spawning period, thinner ovarian wall with follicular epithelium which get separated from each other, are seen. The size of yolk granules has considerably increased in the oocyte. Shrinked nucleus with totally ruptured nuclear membrane were also observed during spawning than control group. Corpora atretica become also shrinked both during pre-spawning and spawning period.

Key words: *Magnesium Chloride, Ovary, Reproductive Cycle, Heteropneustes fossilis (Bloch)*

INTRODUCTION

The present study has been planned to observe the effect of highest magnesium concentrations in the ovary of *Heteropneustes fossilis* (Bloch) during its reproductive cycle. Important work on the structure of the fish ovary alongwith its seasonal changes in the annual reproductive cycle have been described by various workers. Bretscheider and Duyvene de wit[5] have studies on sexual endocrinology of non mammalian vertebrates. Raizada[10] has also studied the reproductive system and the reproductive cycle in some teleosts Rasbora daniconius (Ham). Saksen[12] has studies on Hypothalamo-Hypophysial Neurosecretory system in fresh water fish Glassogobius giuris (Ham.) in relation to reproduction. Yamamoto[18] has studies on the formation of fish egg[1]. Annual cycle in the development of ovarian eggs in the flounder, Liopsetta obscura. Stractral changing in overy has been elaborately described by various workers.

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Baruah B. K&Das m[1] Deshmukh S.V& Kulkarni K.M[6]. Kumar S. & Pant S.C[7].Singh.K[7] Shukla V *et al*[17]. Mishra A.K and mohanty B[8]. Sharma *et al.*[13] have reported Effect of manganese on ovaries of Garra gotlya gotlya. Sharma *et al.*[14] Histological Studies on the candmium chloride exposed Air – Breathing Fish *Heteropneuste fossilis* (Bloch), Mallick A, *et al*[9]. Bioaccumulation of alkali and alkaline earth metals (sodium, potassium. Calcium and magnesium) in fish (Labeo rohita Ham) organs from selected districts of Odisha India.

MATERIAL AND METHODS

The fish *Heteropneustes fossilis* (Bloch) ,were obtained from local Sagar lake, Sagar, M.P. Twenty four adult fishes were collected during the first week of every month for one complete reproductive cycle i.e.; for continuous 12 months. The eyes as well as the surface bones of skull were removed and an incision was given in the abdomen so as to ensure efficient and optimum fixation. During the experimental period the mature fishes ranging between 12 to 17 cm in length and 25-30 gm in weight were placed in tap water aquarium in laboratory conditions and treated with tetracycline to control bacteria and other out breaks in Post-spawning (December), Pre-spawning (April) and Spawning period (July). Healthy fishes were selected for the experimental work. Eight fishes were kept in each aquarium which contains 24 litre tap water i.e. 3 litre/fish. They were acclimatized for about a week before starting the experiment. During this period fishes were fed with dried shrimps. However, they were not fed throughout the experimental period and the water of each aquarium was renewed twice a week.

Experiment with different Magnesium concentrations

The whole set up was planned as described earlier for Calcium exposure. The experiments were set in following way as per the protocol of S.E. Wendelaar Bonga *et al.*[4].

1. Gradually fast transfer in different Megnasium concentrations during post-spawning period (December). The fish *Heteropneustes fossilis* (Bloch) belonging to experimental group were gradually adapted from 10 m mol l-1, 20 m mol l-1, 35 m mol l-1 and 80 m mol l-1 (each step lasted for a day). In 80 m mol l-1 magnesium chloride ($MgCl_2.6H_2O$, E. Merck) solution the animal could not survive for more than 7 to 8 hours and is found lethal.

2. Gradually fast transfer in different Megnasium concentrations during pre-spawning period (April). The whole set up was as described above.

3. Gradually fast transfer in different Megnasium concentrations during spawning period (July). The fish Heteropneustes fossilis (Bloch) belonging to experimental group were gradually adapted from 10 m mol l-1, 20 m mol l-1, 35 m mol l-1 and 55 m mol l-1 (each step lasted for a day). In 55 m mol l-1 magnesium chloride ($MgCl_2.6H_2O$, E. Merck) solution the animal could not survive for more than 7 to 8 hours and is interestingly found lethal at this phase of reproductive cycle

Softening of Yolk in Ovary:

For dehydration of the tissue at 70% alcohol (2% phenyl solution) is used. Keep ovaries in the oven at 35-36^{0}C for a week and wash in 70% alcohol for several times till smell of phenol is completely removed. Blocks were prepared prior to proper sectioning. (Saxena[12]). At the time of sacrifice the fish were killed by a single blow in the head and important cytological details of ovary was dissected carefully and fixed immediately in proper fixative Holland's modified bouin and 70% alcohol it was thoroughly washed dehyadradeted and than embedded in paraffin wax (melting point 60-62^{0}C) suitable sectioning at 5 -6µ were made to prior to specific and suitable staning.

Stanis Used

Following normal stains i.e.Hematoxylin and eosin were used for ovary showing differentiation of various cell types.

RESULTS

MORPHOLOGY AND HISTOLOGY OF THE OVARY

The ovary in the *Heteropneustes fossilis* (Bloch) is an elongated and spindle shaped structure suspended in the coelom by a peritonium fold known as mesovarian in between the kidney and alimentary canal. The anterior portion of the ovary is conelike and tapering, the middle is wider and posterior is narrower. The peritoneal folds of the posterior side of each ovary show an extension of a hollow tube like structure forming the oviduct which ultimately joins posteriorly with the fellow of the opposite side to form a common oviduct and opens to the exterior through the genital pore. The ovaries are enclosed in thin peritoneal covering. The ovarian wall is composed of an outer layer of fibrous connective tissue traversed by the blood capillaries and an inner layer of germinal epithelium. The ovigerous lamellae containing oocytes in various stages of development projected from the ovarian wall towards the centre of the ovary. The early stages of oocytes are found on the periphery of the lamellae. They migrate towards the ovocoel centre during their development. The ovigerous lamellae are covered by a thin layer of epithelial cells.

OOGENETIC STAGES:

Different oogenetic stages in *Heteropneustes fossilis* (Bloch) can be devided into ten developmental stages on the basis of both nuclear and cytoplasmic changes with special reference to vitellogenesis. The different developmental stages as described here are based on the work of Yamamoto[18] which provides enough scope to study the various stages of the ovary as follows:

i. Early chromatin –nucleolus stage
ii. Late chromatin-nucleolus stage
iii. Early peri-nucleolus stage
iv. Late peri-nucleolus stage
v. Early yolk-vesicle stage
vi. Late yolk-vesicle stage
vii. Early yolk stage
viii. Late yolk stage
ix. Pre-maturation stage
x. Maturation stage

CORPORA ATRETICA & POST OVULATORY FOLLICLES:

Corpora atretica:

The immature oocytes which fail to attain maturity and the mature oocytes which fail to spawn ultimately undergo resorption or atresia and are called corpora atretica or atretic follicles. In the process of resorption, granulosa layer or follicular epithelium of the ovarian follicle plays an important role. Bretschneider and Duyvene de with were the first to make an exhaustive study in Rhodeus amarus.Bhargava2 has used the term corpora atretica. They produce the enzymes which digest the yolk, the cytoplasm and the nucleus. The process of atresia of mature oocytes have been distinguished into four different stages.

(a) Stage I
(b) Stage II
(c) Stage III
(d) Stage IV (Fig.3 and 5)

Post-ovulatory follicles

The post-ovulatory follicles (empty or ruptured follicles) are formed after the extrusion of mature oocyte from the ovary. The frequency of the number of post-ovulatory follicles in the ovary is an useful measure to estimate the spawning periodicity of the fish.

Post-ovulatory follicles of Heteropneustes fossilis are convoluted structure containing an irregularly shaped structure. (Fig.3)

Gradual fast transfer in different magnesium concentrations of experimental group during post-spawning period

The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 10 m mol l^{-1}, 20 m mol l^{-1}, 35

m mol l^{-1}, 55 m mol l^{-1} and 80 m mol l^{-1} (each step lasted for a day). In 80 m mol l^{-1} magnesium chloride ($MgCl_2.6H_2O$) solution, the animal could not survive for more than 7 to 8 hours and therefore is found lethal. Important cytological changes were observed during this concentration in ovary. The thick ovarian wall, follicular epithelium and the ovigerous lamellae are in ruptured condition. The nuclear membrane is ruptured and nucleus is shrinked. Early chromatin nucleolus stage is also found in shrinked condition and is basophilic in nature. Late chromatin nucleolus stage is also highly basophilic. In yolk vesicle stage the number of vacuoles has considerably increased. (Fig.4)

Post-spawning period (Control Group)

During this period the ovarian wall is thick and distinct. During control group the ovigerous lamellae contain numerous oogonia. Interfollicular space is still distinct. Late peri-nucleolus and yolk vesicle stage were also observed. Corpora atretica and post-ovulatory follicle are totally absent. (Fig.1)

Gradual fast transfer in different magnesium concentrations of experimental group during pre-spawning period

The fish *Heteropneustes fossilis* (Bloch) belonging to experimental group were gradually adapted from 10 m mol l^{-1}, 20 m mol l^{-1}, 35 m mol l^{-1}, 55.0 m mol l^{-1} and 80 m mol l^{-1} (each step lasted for a day). In 80 m mol l^{-1} magnesium chloride ($MgCl_2.6H_2O$) solution the animal could not survive for more than 7 to 8 hours and is found lethal. Important cytological changes were observed during this concentration in ovary. The thin ovarian wall and the corresponding follicular epithelium are ruptured. Vitelline membrane and follicular epithelium get separated. The size of yolk granules considerably increased. Shrinked nucleus with totally ruptured nuclear membrane was also observed. Late perinucleus stage is basophilic though stained light due to magnesium exposure. The corpora atretica become shrinked in comparison to the control group. (Fig.5)

Pre-spawning period (Control Group) during this period the ovarian wall is thick and highly vascular. The ovarian wall becomes thinner as compared to that of preceding month. The late yolk vesicles stage showed vaculation while yolk formation also started in the cytoplasm. Early yolk stage lies towards the border of the ovigerous lamellae. A few corpora atretica are present. Post-ovulatory follicles are completely absent. (Fig.2).

Gradual fast transfer in different magnesium concentrations of experimental group during spawning period

The fish *Heteropneustes fossilis* (Bloch) belonging to experimental group were gradually adapted from 10 m mol l^{-1}, 20 m mol l^{-1}, 35 m mol l^{-1} and 55 m mol l^{-1} (each step lasted for a day). In 55 m mol l^{-1} Magnesium Chloride ($MgCl_2.6H_2O$) solution the animal could not survive for more than 7 to 8 hrs and is found lethal. Important cytological changes were observed during this concentration in ovary. The thinner ovarian wall alongwith follicular epithelium is ruptured. The follicular and vitelline membrane became isolated. The membrane of mature oocyte become shrinked and the size of yolk granules increased considerably. These granules became degranulated and seem to be dissolved in the cytoplasm. Corpora atretica and post-ovulatory follicle are interestingly found shrinked but not show in figure. Nucleus is in highly damaged condition. (Fig. 6)

Spawning period (Control Group)

During the spawning period, the ovarian wall is very thin a large number of ripe oocytes are present along with few immature oocytes. Their numbers are fewer than in the preceding month. majority of the ova in the ovary become fully ripened and are closely packed together. A very few immature oocyte are also present in between the ripe ones. The corpora atretica and post ovulatory follicles are also seen during this period. (Fig.3).

DISCUSSION

The origin of the new crop of oocytes in faced with divergent opinions and the observations in this direction are variable.Yamamoto[18] state that the new crop of germ cells originates from the follicles. Cells contained in the empty follicle are left behind after the extrusion of the mature oocytes. Raizada[10] has observed that the new oocyte are formed as a result of proliferation of germinal epithelium while Belsare[3] has noticed the origin of new crop of the oocytes take place from the pre existing oogonia. Saxena[12] has suggested that the new crop of oocytes is derived from the germinal epithelium. In *Heteropneustes fossilis* it has been observed that the new oogonia appear from the germinal epithelium and transformed into oocyte during a short rest period i.e. the early chromatin-nucleolus stage is always found associated with the germinal layer. Siosion and Herrera[16] also reported a reduction in number and size of oocytes in the ovaries of fishes colisa fasciatus, oreochromis massambicus and *Heteropneustes fossilis* (Bloch). Following exposure to metals like nickel and cadmium chloride. Mishra and mohanty[8] also reported large interfollocular spaces in ovaries due to shrinkage of oocytes under the effect of metal toxicity. Mishra and mohanty[8] also reported necrotic and vacuolated oocytes in the ovaries of fishes .Sharma *et al*.[13] have reported that the effect of Manganese on ovaries of Garra gotlya gotlya the reproductive potential (by reducing in fecundity) as evident by reduction in number and size of oocytes and increase in atretic oocytes. Sharma et al14 have reported long term exposure of cadmium chloride in *Heteropneustes fossilis*. Resulted in marked degenerative hanges in the ovary. These changes included prominent interolliculler spaces. Kumar and pant7 reported a singnificant atresia in the ovary with major damage to younger oocytes in puntius conchonius, after exposure to zinc on gonads. Baruah and Das1 also reported partial lysis, swelling and change in nucleus after exposure of 20 days. Paper mill effluent. Shukla *et al*.[17] have reported histological and biochemical effects of heavy metals on the ovary.

In Fish *Heteropneustes fossilis* (Bloch) highest Magnesium exposure of experimental group, thick ovarian wall and ovigerous lamellae are in ruptured condition in comparison to control group. But early chromatin nucleolus and late chromatin nucleolus stage are found highly basophilic than control during post-spawning period. During pre-spawning and spawning period, thinner ovarian wall with follicular epithelium which get separated are seen. The size of yolk granules has considerably increased than control. Shrinked nucleuswith totally ruptured nuclear membrane were observed than control group. Corpora atretica become shrinked during pre-spawning and spawning period. It can be concluded that highest magnesium exposer shows acute stress and such changes adversely effect histology of ovary and fish reproduction.

Fig. 1 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) in Control group during post-spawning period (December) Showing late Peri-nucleolus and early yolk vescle stage.
H & E 60x

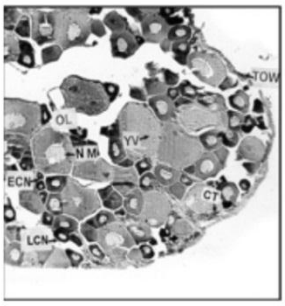

Fig. 4 Photomicrograph of T.S. of the Ovary,of *Heteropneustes fossilis* (Bloch) Showing the effect of 80 m mol 1-1 of magnesium chloride (Mgcl2.6H 2O) Solution during post-spawning period (December)
H & E 60x

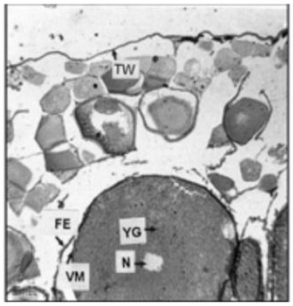

Fig. 2 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing Control group during pre-spawnin period (April)
H & E 60x

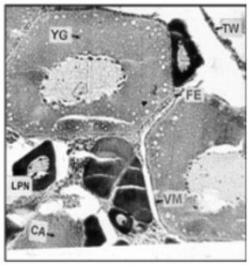

Fig. 5 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) Showing the effect of 80 m mol 1-1 of magnesium chloride (Mgcl2.6H 2O) Solution during pre-spawning period (April)
H & E 60x

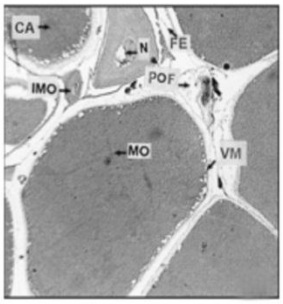

Fig. 3 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) Showing Control group during spawning period (July)
H & E 60x

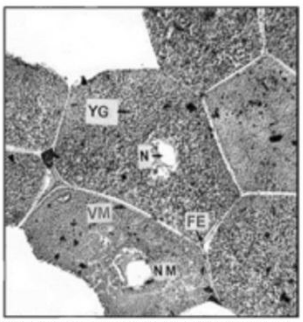

Fig. 6 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) Showing the effect of 80 m mol 1-1 of magnesium chloride (Mgcl2.6H 2O) Solution during spawning period (July)
H & E 60x

C.T. : Connective,E.PN. : Early Peri-nucleolus,E.YN. : Early Yolk vesicle stage, L.PN : Late Peri-nucleolus stage, O.L. : Ovigerous Lamellae T.O.W. : Thick Ovarian wall, C.A. : Corpora atretica F.E. : Follicular epithelium, L.PN. : Late Peri-nucleolus stage, T.W. : Thin Ovrarian wall V.M. :Vetelline membrane,Y.G. : Yolk granules,P.OF : Post-ovulatory follicle, N.M. : Nuclear membrane, N : Nucleus,

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