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Full Length Research Paper

Effects of reproductive biology on heavy metal pollution on the histopathological structure of gonads in India

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Water pollution caused by heavy metals affects breeding and development in fishes of Harike wetland. The effects of heavy metals on fishes are related to their uptake and accumulation by the organism, resulting in metal induced disturbances in the structures and functions of various tissues and organs. Early life stage of fish development, such as oocytes maturation is very sensitive to intoxication. Samples of the fish *Labeo rohita* were collected from the two sites to assess the effects of the water quality and concentrations of heavy metals on the fish ovary. The histopathological changes in gonads have been studied due to exposure of different pollutants. It was concluded that incidences of gonadal abnormalities in the form of deformed oocytes, reduction in their numbers and lack of active oogenesis have been observed. From the results it is inferred that wetland is passing through an alarming situation because deformities in early stages of oocytes have been observed. It is posing a serious threat to the biodiversity existing there. It is recommended that waste water discharge from various sources should be treated to protect the fish and the public health from the menace of pollution.

Key words: Wetland, Industrial pollution, toxicity, *Labeo rohita*, oocytes.

INTRODUCTION

Heavy metals are the major source of water pollution, as it eradicates the economically important species either indirectly through breaking the biological chains or directly produces toxic stress by means of chemical changes in water. As a result, large scale mortality of fishes has been observed due to discharge of heavy metal pollution into natural water resources (Srivastava and Srivastava, 1994). Fishes are very sensitive to a wide variety of toxicants in water, various species of fish uptake and accumulate many toxicants such as heavy metals (Herger et al., 1995). The accumulation of heavy metals in tissues thus causes many physiological, histological and biochemical changes in the fishes and other freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma and Ramamurthi, 1982).

Figure 1. Collection sites at River Beas and Harike wetland.

The pervious histopathological studies of fish exposed to pollutants also revealed that fish organs uptake toxicants and are efficient indicators of water quality (Cardoso et al., 1996; Cengiz et al., 2001; Benejam et al., 2010). The effects of different toxicants on the aquatic fauna, particularly on fish have received attention of many investigators (Das and Baruah, 2002; Athikesavan et al., 2006; Gupta and Srivastava, 2006; Tilak et al., 2007).

The main objective in aquaculture is to enhance fish production by producing high numbers of viable oocytes in controlled conditions and subsequently to ensure the development of normal embryo (Lubzens et al., 2010; Bobe and Labbe, 2010). Nevertheless in natural aquatic ecosystems, environmental toxicants discharged from various sources causes detrimental effects on important features such as metabolism, growth, reproduction and ultimately the survival of the fish becomes very difficult (Adams et al., 1992; Benejam et al., 2008). For further propagation of the fish, the quality of oocytes plays a key role in the proper development of an embryo. The competence of oocytes depends on numerous processes taking place during the whole oogenesis, but its final steps such as oocyte maturation, seems to be of key importance (Jezierska et al., 2001; Burger and Gochfeld, 2005; Marteil et al., 2009; Ebrahimi and Taherianfard, 2011). The toxicological effects on reproduction in the wild fish have been barely investigated with regard to alterations in the gonads (Adams and Greely, 2000; Jobling et al., 2002; Toft et al., 2004). Hence, the present investigation was undertaken with a view to study in detail about histopathological changes in the ovary of *Labeo rohita*, under the influence of heavy metal toxicity and to assess the extent of damage in this wetland.

MATERIALS AND METHODS

The present study was conducted from March 2013 to February 2014 during different seasons. Two sites (Figure 1) were chosen to carry out present study; the first one was located in River Beas at Marrar village, latitude 31°09'55.08"N and longitude 74°57'38.40"E (used as the reference point) and the second was selected in Harike wetland, latitude 31°08'42.08"N and longitude 74°59'31.47"E.

Fish sample collection and histopathological examination

Five samples of Indian Major Carp *L. rohita* were collected from each site during breeding season. The fishes were dissected on the spot for histological studies. After dissecting the fish, ovaries were removed and fixed in Bouin's solution for 24 h. The tissues were routinely dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 4 to 6 µm thick were cut, processed and stained with heamatoxylin and eosin (H&E). They were examined and photographed under light microscope unit.

RESULTS

Histopathological findings

The histological examination of the ovaries of *L. rohita* from river Beas (Figures 2 to 6) showed that in early stage of oocyte maturation the ovary is mainly composed

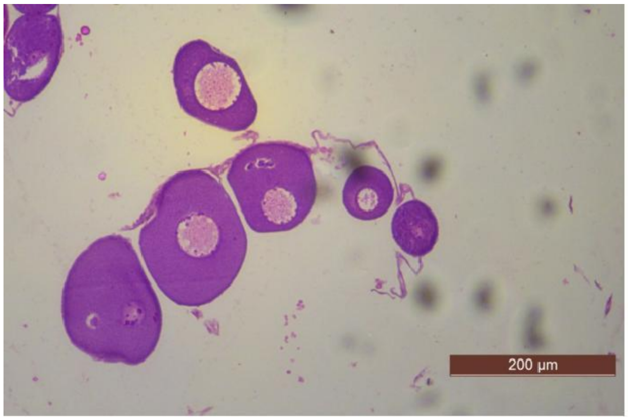

Figure 2. Micrograph showing different stages of oocytes in ovary of *Labeo rohita* from River Beas or control group.

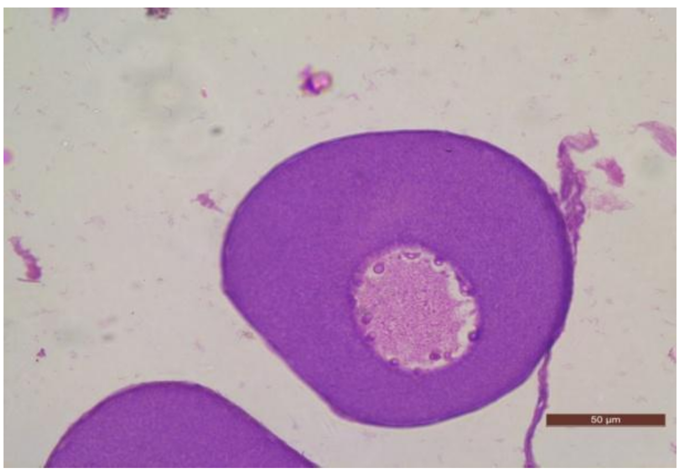

Figure 3. Periucleoar stage of oocyte.

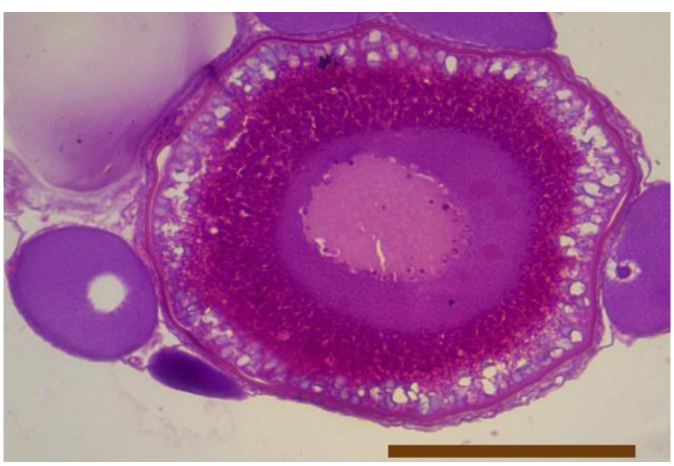

Figure 4. Cortical alveolus stage of control group with demarcation of zona radiata and germinal vesicle migration stage of oocyte.

of small oogonia (Figure 2) and this stage is called chromatin nucleolus stage of oocyte maturation.

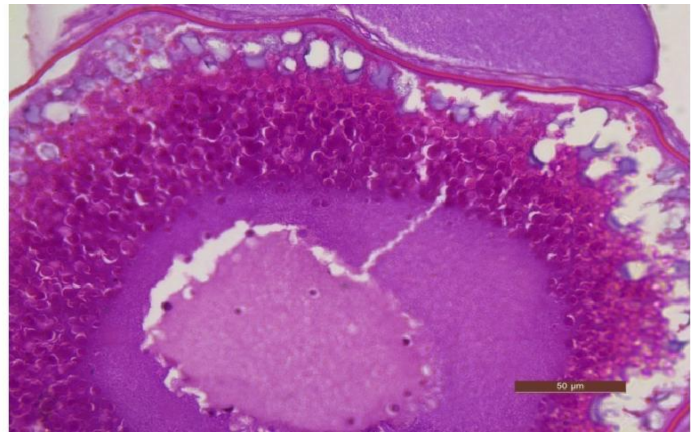

Figure 5. Micrograph showing cortical alveolus stage of oocytes.

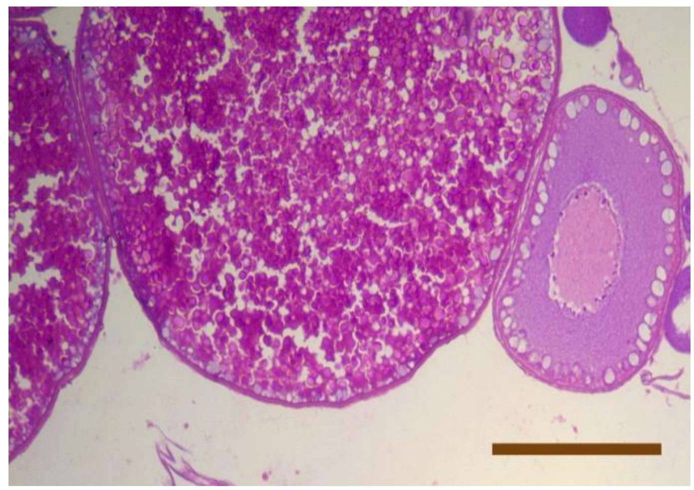

Figure 6. Vitellogenic stage of oocyte and privitellogenic stage of oocyte in control group.

The perinucleolar oocytes are enlarged in size with undifferentiated membrane, the nucleoli increased in number and become close to the nuclear membrane (Figure 3). On further maturation, the oocytes entering yolk vesicle stage in which deposition of yolk and fat globules starts which increase the size of maturing egg. The oocyte membrane at this stage became well developed (Figure 4). The cytoplasm loses its basophilic nature and became fully occupied with yolk granules. At vitellogenic stage the vacuoles become connected to each other and nucleus began to liberate its substances into cytoplasm and the nucleus starts to migrate to the animal pole (Figure 5). In mature egg, nucleus also loses its membrane and merged entirely into the cytoplasmic inclusion and moves towards the animal pole, liquefied yolk material is distributed throughout the cytosome (Figure 6).

On the other hand fishes collected from Harike wetland (Figures 7 to 13) showed different histological forms. Atretic oocytes with broken membrane which invade the

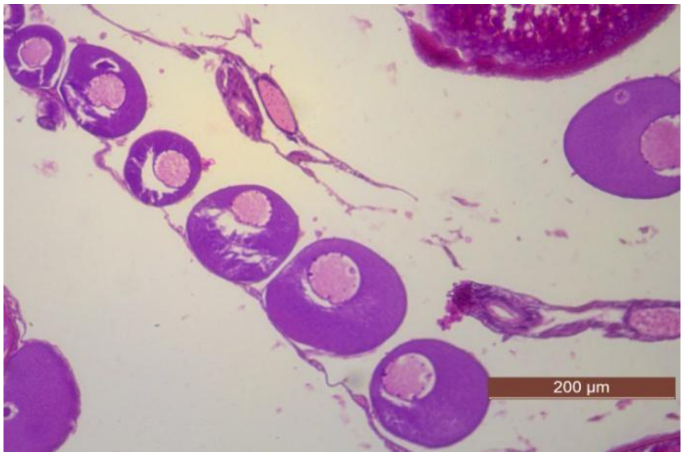

Figure 7. Atretic oocytes, decreased nucleoli in perinucleolar stage.

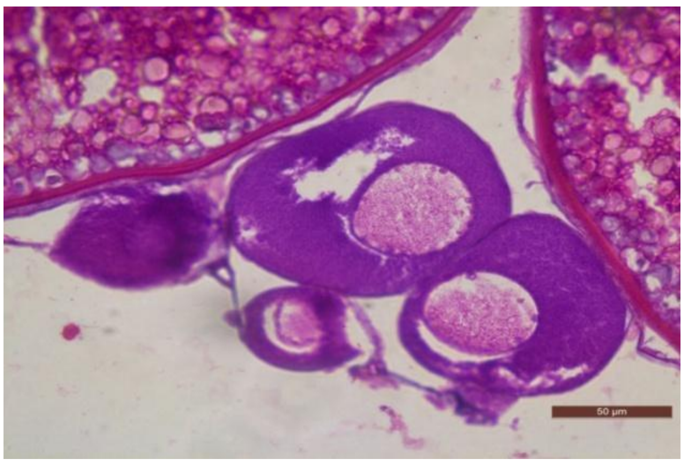

Figure 8. Atretic oocytes and altered chromatin nucleolar stage of oocyte development.

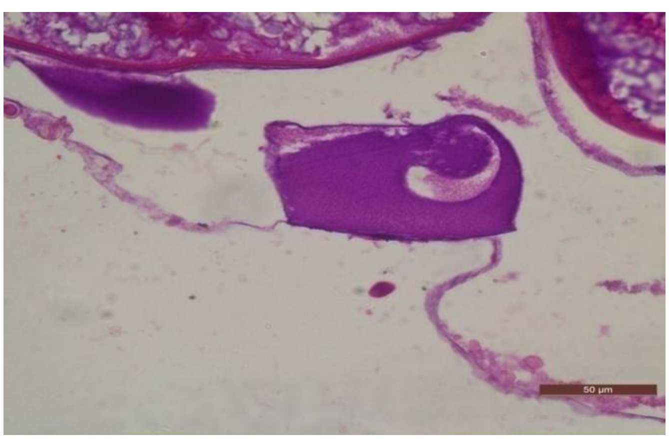

Figure 9. Atretic oocytes in ovary of fish.

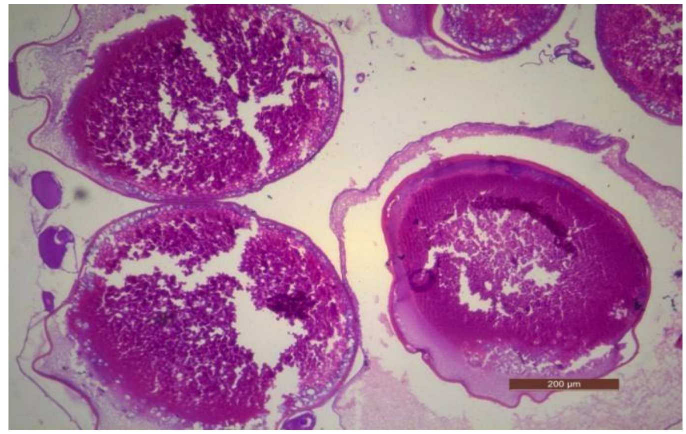

Figure 10. Development of interafollicular space in oocytes and dissolution of oocyte wall.

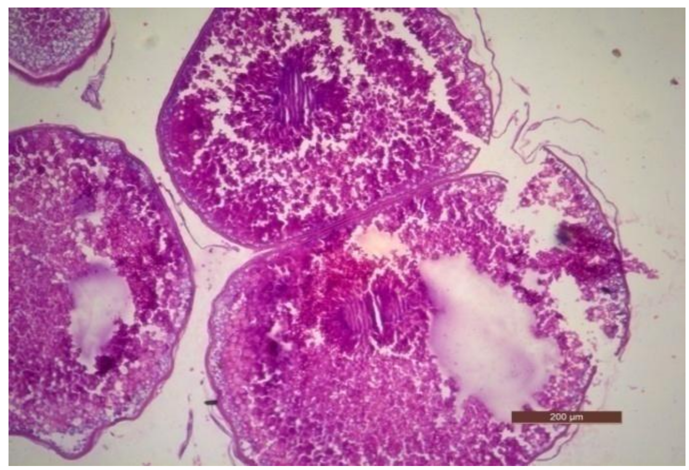

Figure 11. Vacuolation and broken wall in vitellogenic oocytes of fish.

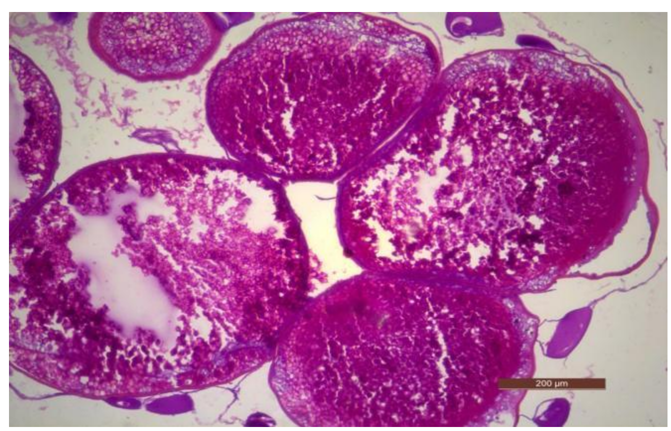

Figure 12. Dissolution of yolk globles and vacuolation in vitellogenic oocytes.

dead ova and vacant space in the ovary (Figure 7). Atresia was seen in the maturing follicles, ovarian follicle separated due to loss of inter-follicular connective tissue, inter follicular spaces were larger and vacuolation in developing oocytes were also observed (Figures 8 and 9).

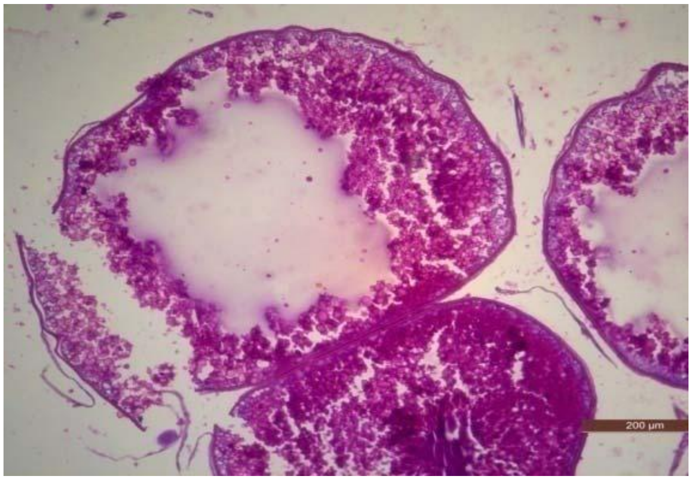

Figure 13. Damage to yolk vesicle and clumping of cytoplasm in mature oocytes.

Development of interafollicular space in oocytes and dissolution of oocyte wall was also observed (Figure 10). Vacuolization in the cytoplasm of the vitellogenic oocytes was observed (Figure 11). Dissolution of yolk globules and vacuolation in vitellogenic oocytes of fish is shown in Figure 12. Damage to yolk vesicle clumping of cytoplasm of maturing oocytes was observed too (Figure 13).

DISCUSSION

Histological abnormalities in ovaries may be caused by several factors, viz., parasitic infection, mechanical injuries, ionizing radiations, by a variety of toxic effluents and aquatic pollutants resulted in liquification of perinucleolar cytoplasm and condensation of nucleus, disappearance of nuclear membrane, cytoplasmic clumping and atretic oocytes (Kling, 1985; Sukumar and Karpagaganpathy, 1992; Sakthival and Gaikwad, 2001; Abou-Seedo et al., 2003; Deshmukh and Kulkarni, 2005; Olfat and El-Greisy, 2007). Similar abnormalities were observed during present investigations.

The different pollutants such as industrial and agricultural wastes, pesticides and heavy metals have histopathological effects on the reproductive tissues of fish gonads (Johnson et al., 1991; Lye et al., 1998; Pedlar et al., 2002; Hanna et al., 2005), these effects may disturb the development of germ cells and may reduce the ability of the fish to reproduce (Inbamani and Seenivasan, 1998; Kumar and Pant, 1984; Mehanna, 2005). In the present study, higher incidence of oocyte atresia was found in the area of Harike wetland than River Beas. Jobling et al. (2002) stated that, atresia was recorded in roach living in rivers that receive treated sewage effluents. Johnson et al. (1997) reported that atresia is thought to be an uncommon event in healthy females and it has been linked to poor nutrition and environmental stress. During the present course of work, it is observed that numbers of mature oocytes have been reduced with large follicular spaces, these observations coincides with those recorded by Unal et al. (2007), Abou Shabana et al. (2008), Mazrouch and Mahmoud (2009), Shobikhuliatul et al. (2013) and Masarat et al. (2014). From this study, it could be concluded that deformed and infected gonads of *L. rohita* collected from Harike wetland area were found in a higher percentage than those of River Beas. These results confirm the presence of different types of pollutants and heavy metals in the water of this wetland.

Conclusion

The changes induced by heavy metal pollutants in fish oocyte maturation may be related to intoxication of eggs, accumulation of metals in eggs or a direct effect of metal on the oogenesis process. The oocyte maturation is the most sensitive to metal intoxication. Therefore, various disturbances induced by heavy metal pollutants during development of oocyte results in a reduced quantity and quality of eggs. Hence, toxicity of wetlands can pose a big threat to the unique biodiversity existing there.

Conflict of Interest

The authors have not declared any conflict of interest.

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