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Histopathological and Histochemical Changes in the Oocytes of the Air-Breathing Fish *Heteropneustes fossilis* (Bloch) Exposed to Textile-Mill Effluent

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Studies on paper-mill and textile-mill effluents discharged into the River Tambaraparani (Tamil Nadu, India) are of great interest to all concerned with water quality in these waters primarily because of high potential input of suspended solids (Sprague and McLeese 1968) and oxygen consuming materials (Johnson 1977). Hence, in our previous investigations we have studied the effects of industrial effluents on histopathology (Haniffa and Sundaravadhanam 1984), haematology (Murugesan and Haniffa 1985) cytopathology (Murugesan et al.1989) and food utilization (Haniffa and Murugesan 1991) of fishes. Literature on histopathological and histochemical changes brought out by industrial effluents on the reproductive tissues, especially the ovary are lacking. Hence this aspect was studied in Heteropneustes fossilis, the most common air-breating fish of the River Thambaraparni.

MATERIALS AND METHODS

Healthy H.fossilis (18 ± 2 g) collected from unpolluted ponds of Palayamkottai and maintained at 28 ± 2° C were used for the present study. Combined textile-mill effluent was collected from Madura Apparel Fabrics situated on the bank of River Tambaraparani (8° 42' N and 77° 24' E, Pabanasam), India. Static bioassay studies were conducted in troughs of 40 L capacity by exposing 10 individuals, in triplicate, to each concentration by providing 2 L of test medium per g of fish (Sprague 1973). For histopathological studies, 15 female test fish were exposed to 3% and 7% effluent concentrations and a control using dechlorinated tap water (pH 7.5 ;

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total hardness ($CaCo_3$) 107 mg/L; total alkalinity 86 mg/L; DO 4.45 mg/L) was maintained and were fed on formulated feeds regularly. Fresh test media were supplied daily and the regime was conducted up to 120 days. Bits of ovary were excised from 3 fishes per concentration and fixed in Zenker's fixative after 7,15,30,60 and 120 days of exposure.

Zenker's - fixed tissues were processed and double embedded in celloidin paraffin (Peterfi's method; Drury and Wallington 1967) and 6 to 7 µm sections were stained for histopatholgy in Weigerts' haematoxylin - Biebrich scarlet (dichrome) and Masson's haematoxylin - Xylidine ponceau - fast green (trichrome) stains. Acrolein - Schiff reaction (Van Duigin 1961) for protein, toluidine blue stained at pH 4.6 for RNA (with or without prior digestion in RNAase), azure B (Flax and Himes 1952) for DNA and RNA have been employed for histochemical localization of these substances.

RESULTS AND DISCUSSION

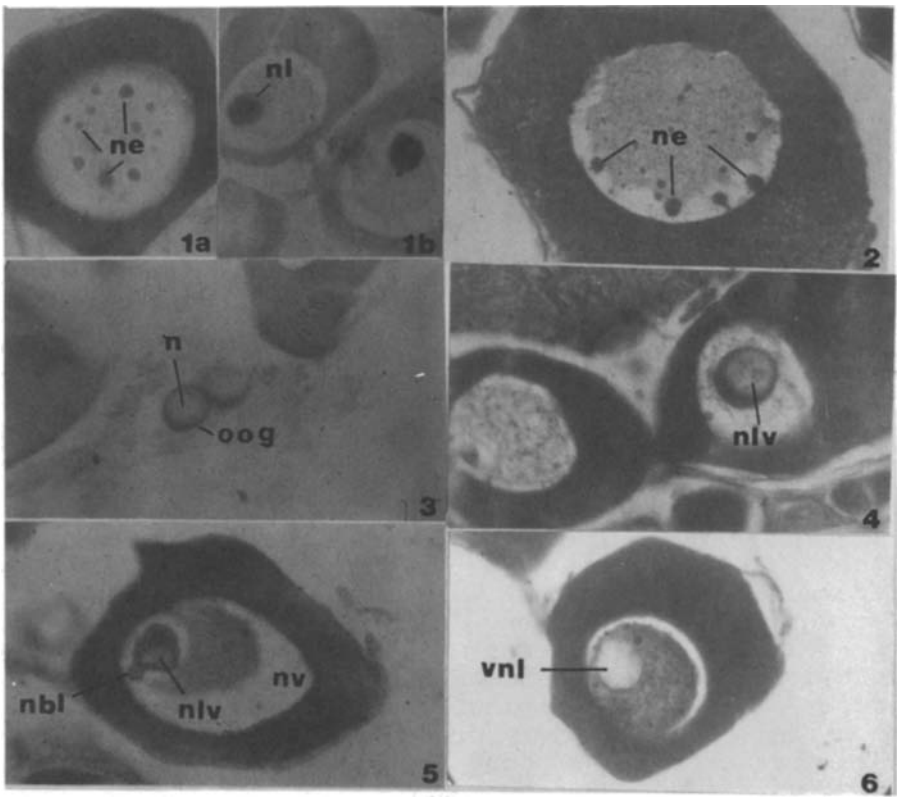
In the control fish, the ovary showed active oogenesis and oogonia were small with prominent nuclei and nucleoli. The primary oocyte in the very early stage had subspherical or oval outline. The cytoplasm was intensely stained and was rich in RNA (Fig. 1a); concomitantly protein synthesis was also high. The nucleus was spherical, with a smooth outline, and the chromosomes, in diplotene stage, had lampbrush organization. One or two prominent, spherical, intensely - stained basiphil bodies were visible within the nucleus; these are nucleoli and contained rich RNA. The chromatin material, being diffuse, was scarcely stainable (Fig.1b). Besides the true nucleoli numerous smaller and slightly irreguler bodies of different sizes were found in the nuclear space (Fig.2). These are nucleolar extrusions (Viswanath 1968) of small refractile masses containing ribonucleoprotein material sloughed off from the loops of lampbrush chromosomes (White 1973) and have a role in protein synthesis and are erroneously described as nucleoli. As the oocyte grows, basiphil substance condenses adjacent to the nucleus in the cytoplasm and this spherical body forms the RNA rich yolk nucleus (see Fig.11) which helps in synthesis of yolk proteins and even yolk lipids. Both within the nucleus and in the cytoplasm there was apparent homogeneity in the control oocyte, there being no vacuoles.

In the early stages of oogenesis, the effect of effluent was most spectacular that the nuelei of the

oogonia underwent complete karyolysis and resulted in the disappearance of the chromatin reticulum and the nucleoli (Fig.3). In the oocyes, the most striking vacuolation, has been consistently observed within the nucleoli and numerous small vacuoles found in some nucleoli produced a frothy appearance. In most of the nucleoli, the vacuoles were centrally placed and were surrounded by a basihil cortex (Fig.4). In certain cases, the nucleoli appeared crescentic with a crater-like depression on one side and a spherical vacuole in the middle. Also, some of the vacuolated nucleoli underwent blebbing (Fig.5). In extreme cases, the entire nucleolus was absent (Fig.6) and there was a large vacuole in its place. The vacuolated region was completely devoid of any RNA. Acrolein - Schiff reaction showed lesser amount of protein in the cortical region of nucleoli of treated fish.

In the oocytes of treated fish, one of the changes frequently observed was the vacuolation in the nucleolar extrusions which appeared as frothy structures with very thin stainable material surrounding the several vacuoles. In addition, the nucleolar extrusions became lesser in number and also their size became diminutive; the larger among them occupied the periphery of the nuclear space and the smaller extrusions occupied the central part of the nucleus. One more characteristic change was vacuolation surrounding the extrusions and each one of the extrusions lied within a vacuole in the nucleoplasm (Fig.7). Histochemically, the extrusions showed loss of basiphilia due to decreased RNA-content (Fig.8). When compared to the normal amount of RNA present in the extrusions, the affected ones showed about 1/4th to 1/6th of the intensity of RNA stain. In extreme cases, the complete absence of nucleolar extrusions was noted. The vacuolation of the nucleus is a prominent pathological change observed and the nucleoplasm occupied only a part of the nuclear volume (see Fig.11).

The most conspicuous change in the cytoplasm was the vacuolation (Fig.9). In addition, the RNA - content of the cytoplasm had also decreased on account of exposure; consequently the content of protein in the cytoplasm was very much lower than that of the normal oocyte (Fig.10). The shrinkage of the nucleus resulted in a vacuole in the cytoplasm. In several oocytes, the size of the yolk nucleus was diminished and was surrounded by distinct vacuole (Fig.11). In extreme cases, the whole yolk nucleus has disappeared leaving just a spherical vacuole. The drastic effect



All figures are transverse sections of the ovary fixed in Zenker's fixative.

Fig.1a. Young oocyte of control fish(120 day) stained in azure B showing intensely stained RNA- rich nucleolar extrusion (ne) and cytoplasm. x 800.

Fig.1b. Control oocyte (120 d) showing positive reaction to Acrolein Schiff and the RNA rich fibrous core of the nucleoli(nl) not stained by Acrolein Schiff. x 800.

Fig.2. Control oocyte (60d; Weigert's haematoxylin - Biebrich scarlet) showing intense basiphilia of cytoplasm and nucleolar extrusions (ne). x 800.

Fig.3. Very young oogonial cells (oog) of fish exposed for 30 d in 3% effluent (toluidine blue) showing complete lysis of the nucleus(n) including nucleolus. x 800

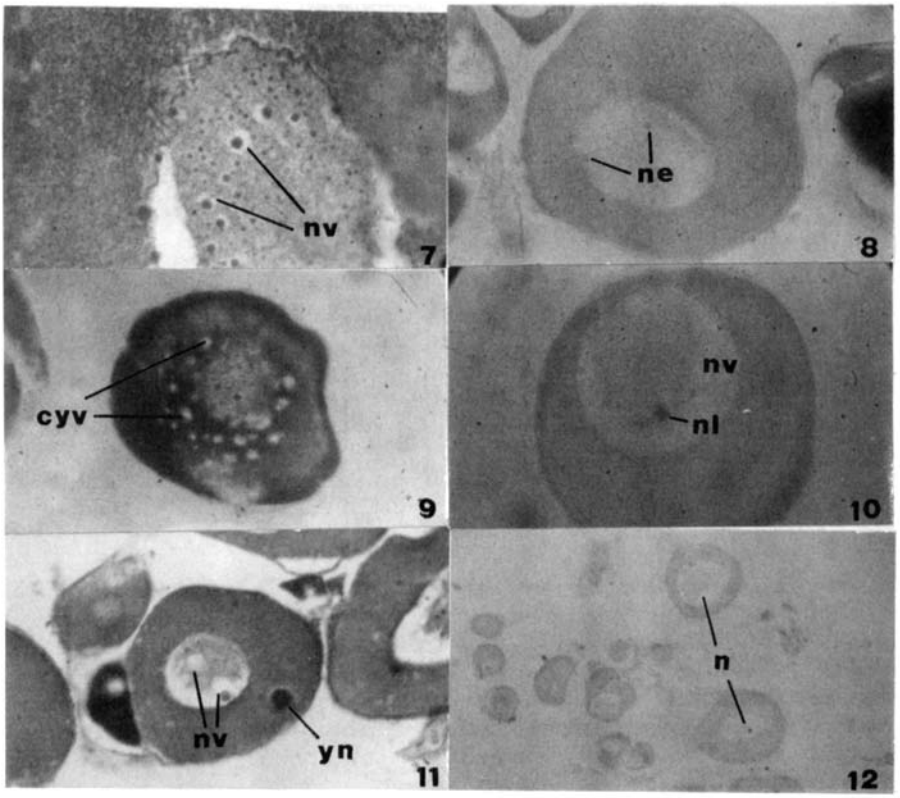


Fig.4. Oocyte (15 d in 3%; Masson's trichrome) showing the conspicuous vacuole in the nucleolus (nlv) surrounded by a basiphil cortex.

Fig.5. Oocyte (15d in 3%; Masson's trichrome) showing large vacuole in the nucleus(nv); the nucleolus shows blebbing (nbl) and a large vacuole (nlv) in the middle. x 800.

Fig.6. Oocyte (120 d in 7%; Masson's trichrome) showing large vacuole in the place of nucleolus (vnl). x 800.

Fig.7. Oocyte (60 d in 3%; Masson's trichrome) showing distinct vacuoles surrounding nucleolar extrusions (nv). x 800.

Fig. 8. Oocyte (60 d in 3% ; Azure b) showing loss of basiphilia of cytoplasm and nucleolar extrusions(ne). x 800.

Fig.9. Oocyte (60 d 7% ; Masson's trichrome) showing several small vacuoles in the cytoplasm (Cyv). x 800.

Fig.10. Oocyte (30 d in 7%); Acrolein Schiff reaction) showing decreased protein content in the cytoplsam and the nucleolus (nl) is very small. Nuclear vacuole is seen (nv). x 800.

Fig.11. Oocyte (60d in 3%; Massons' trichrome) showing a small yolk nucleus(yn) surrounded by a vacuole. Nucleus shows vacuolation (nv). x 800.

Fig.12. Very young oocyte (60d in 7%; Acrolein - Schiff reaction) showing pronounced decrease in protein content, small nucleus (n) and absence of nucleolar extrusions. x 160.

of the effluent was seen even in early oocytes; the entire oocyte showed very pronounced decrease of protein - content in the cytoplasm as well as the nucleus. Such oocytes were bound to become atretic (Fig.12). In general, the changes produced were pronounced as the concentration and duration of exposure increased.

Cameron (1964) stated that vacuolation indicated the onset of cytopathological changes; the flux of electrolytes through the plasma membrane is greatly affected. As a result the control exerted by the plasma membrane in ion-transport is lost to a very great extent. Usually, such cells show an increase in the volume, which is sometimes described as hypertrophy and it becomes necessary for the extra space to be vacuolated as the protoplasm cannot occupy the increased volume.

Vacuolation of the nucleolus and its ultimate atrophy are not explained, even if recorded, by pathologists. This requires a cytological explanation based on nucleolar ultrastructure; the nucleolus comprises a core of fibrous part (pars fibrosa) surrounded by a cortex of granules (pars granulosa). These are embedded in the pars amorpha. The pars fibrosa is composed of RNA; the pars granulosa contains protein as well. The nucleolus, being the structure where ribosomes are assembled, contains almost all the nuclear RNA. The basiphilia of te nucleolus depends on its RNA content. The constituents of the effluent

affect RNA synthesis in the oocytes; as a result, there is a loss of basiphilia of the nucleolus as a whole. When there is drastic effect of effluent, RNA synthesis comes to a standstill and the pars fibrosa is absent; in its place there is a vacuole which is visible as a clear space in histological and histochemical preparations. As a consequence of the absence of RNA of pars fibrosa, the assembly of the ribosomes also becomes affected; in such cases even the pars granulosa is absent and there is no nucleolus at all. Thus, ribosomes are no more produced by nucleolus; as a chain reaction this affects protein synthesis by the cytoplasm which comes down to very low levels as indicated by histochemical observation. The effluent constituents (mg/l in the undiluted effluent) : copper 7.6%; chromium 4.8; zinc 6.9; sodium 480; sulphate 520; chlorides 1617; total alkalinity 286; total solids 2692; BOD 572 (Murugesan 1988) have deleterious effect on the organelles which compose the yolk nucleus; this resulted in a reduction in its size or its ultimate obliteration and the oocytes cannot synthesize the nutrients in the absence of yolk nucleus.

The nucleolar extrusions are nothing but mRNA elaborated by lampbrush loops. The diminished size of the extrusions, the vacuolation of these bodies, the decrease in their number and their complete absence indicate the extend to which mRNA synthssis are the casualities of the effect of effluent. It is possible to suggest that the chronic exposure to the textile-mill effluent may be altering the level of gonadotropins either directly and / or indirectly since its titures pricipally regulate the process of gonadial recrudescence in fishes.

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