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# Histopathological Changes in the Gonads of *Oreochromis niloticus* Exposed to Zinc and/or Cadmium

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**Abstract** The histopathological changes in the ovaries and testes of a freshwater teleost, *Oreochromis niloticus* were studied following exposure to zinc and/or cadmium for a period of 7, 15 days. In the ovaries, the histopathological changes included separation and degeneration of the ovarian wall, degenerative and necrotic changes (atresia) in oocytes, shrinkage with distorted appearance of oocytes, separation of the follicular layers from the oocytes, proliferation in the granulosa layer of oocytes, lysis of the yolk granules of mature oocytes, liquification of the cytoplasm of oocytes as well as focal areas of necrosis in the ovarian stroma. Moreover, intravascular haemolysis and dilation in the ovarian blood vessels were observed. Vitellogenic fluid was seen in the ovarian cavity. In the testes, hypertrophy with vacuolar degeneration in the spermatogonia, degenerative and necrotic changes in the cellular elements of the seminiferous tubules, focal areas of necrosis, degeneration in the wall of seminiferous tubules as well as in the interstitial cells and atrophy in seminiferous tubules were observed. The overall structure of testes looked disrupted. It was concluded that exposure to Zn and Cd cause marked histopathological changes in the gonadal structural which could seriously affect the reproductive potential of fish.

**Key words:** Zinc · Cadmium · Ovary · Testis · Histopathology · Fish · *Oreochromis niloticus*

## INTRODUCTION

Environmental pollution and its effects on the health of aquatic ecosystems is a great problem that has been studied intensely in the last years. Intense activity in industrial and agricultural sectors has inevitably increased the levels of heavy metals in natural water [1,2]. Heavy metals are serious pollutants of the aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organisms [3]. Zinc is an essential micronutrient involved in a wide range of biological processes including enzyme catalysis and protein structure [4,5]. However, at high concentrations, Zn exerts adverse effect in fish by inducing structural damage, which affects the growth, development and survival of fish [6]. Cadmium, a non-essential heavy metal, is a non-degradable cumulative pollutant. It is one of the most deleterious xenobiotic in aquatic ecosystems. The harmful effects of Cd included hepatic and renal damage and testicular atrophy [7,8].

According to Gerking [9] tolerance to stress is likely to be lower in the reproductive tract than in any other organs in fish. It has been reported that heavy metals affect both quality and quantity of the gametes as well as the endocrine system, disrupting the gametogenesis [10]. Mechanisms of these harmful effects depend on the type of metal and its concentration. Histopathological alterations in the gonads of fish induced by exposure to different heavy metals have been reported by several authors [11-14]. Hanna *et al.* [15] reported that lead and chromium induced congestion of blood vessels, atrophy in the seminiferous tubules, vacuolation and necrosis of the spermatocytes, a decrease in the numbers of sperms in the lumen of seminiferous tubules as well as vacuolation and necrosis of the germinal epithelium of testes of *O. niloticus*. Moreover, there was a decrease in the numbers of mature ova and most of them appeared atretic in ovaries of *O. niloticus* exposed to lead and chromium [15]. Liao *et al.* [16] observed thickening of the spermatogenic tubule walls, clusters of dead

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spermatozoa in remaining sperm cysts and a hyperplasia of fiber in tubule walls in the testes of *O. latipes* exposed to methylmercury. Yamaguchi *et al.* [17] reported that lead and arsenic caused necrosis of spematogonia and might inhibit spermatogenesis in Japanese eel. In the ovaries of *C. gariepinus* exposed to lead, diffuse mild degeneration and necrosis of the follicles were initially observed, subsequently, marked severe degeneration of the ovarian follicles were seen [18]. Mishra and Mohanty [19] observed an increase in the percentage of atretic oocytes and a decrease in the percentage of vitellogenic oocytes in the ovaries of *C. punctatus* exposed to chromium. Verma and Srivastava [20] noted shrinkage of oocytes, which resulted in large interfollicular spaces, distorted appearance of oocytes and increase in number of atretic follicles in the ovaries of *C. punctata* exposed to zinc.

The aim of the present study was to investigate the possible histopathological changes in the ovaries and testes of *O. niloticus* exposed to Zn and/or Cd.

## MATERIALS AND METHODS

Healthy specimens of *O. niloticus* with average body weight of 56.86±9.30 g and average length of 15.09±1.92 cm were obtained from a fish farm at Al-Kanater Al-Khairya. Fish were acclimated to the laboratory condition for 15 days in large fiberglass tanks containing well aerated tap water (temperature, 24±1°C, pH, 7.2±0.1 and oxygen concentration, 7.8±0.9mg/l). During acclimation, the fish were fed on commercial pellets (28% protein) once per day. Water was renewed after every 24h with routine cleaning of the aquaria, leaving no faecal matter or unconsumed food. Two days prior to application of zinc and cadmium, the fish were transferred from the stock tanks to glass aquaria (100L) provided with well aerated tap water.

Based on the results of a previous study [8] the median lethal concentration at 96h (96h-$LC_{50}$) was 11.25mg/l for Zn and 7.25mg/l for Cd. Seven groups of 30 fish were used. Group 1: exposed to1.125mg/l (10% of 96h-$LC_{50}$) of Zn, Group 2: exposed to 2.250mg/l (20% of 96h-$LC_{50}$) of Zn, Group 3: exposed to 0.725mg/l (10% of 96h-$LC_{50}$) of Cd, Group 4: exposed to 1.450mg/l (20% of 96h-$LC_{50}$) of Cd, Group 5: exposed to mixture of 1.125mg/l of Zn+0.725mg/l of Cd, Group6: exposed to mixture of 2.250mg/l of Zn+1.450mg/l of Cd, Group 7: served as control. Feeding was allowed in the experimental as well as control groups every day for a period of 3h before renewal of the medium throughout the period of the experiment. Both treated and control fish were sampled for histological investigations after 7 and 15 days of exposure. After dissection of fish samples, tissues specimens from ovaries and testes were taken and fixed in10% neutral-buffered formalin. The material was dehydrated, embedded in paraffin wax and cut in 4-6μm thick sections then stained with haematoxylin and eosin according to the method described by Bernet *et al.* [21].

## RESULTS

### Histopathological Alterations in the Ovaries

**Control Ovaries:** The ovaries of fish from the control group showed normal histological structures (Figs. 1a,b).

**Ovaries of Fish Exposed to 1.125mg/l Zn:** The ovaries of fish exposed to 1.125mg Zn/l for 7 and 15 days showed degenerative and necrotic changes (atresia) in oocystes (Figs.1c-h), shrinkage with distorted appearance of oocytes (Figs.1i-k), focal areas of necrosis in the ovarian stroma (Fig.1l), haemorrhage between the oocytes (Fig.1m), dilation of the ovarian wall blood vessels (Fig.1n) and proliferation in the granulosa layer of oocytes, resulting sometimes in adhesion in the cellular coat of the oocytes (Figs. 1o,2a). Separation of the follicular layers from the oocytes (Figs. 2b,c) as well as separation of the ovarian wall (Figs.2d,e) were seen. The ovarian lesions were more severe in fish exposed to 1.125mg Zn/l for 15 days.

**Ovaries of Fish Exposed to 2.250mg/l Zn:** In fish exposed to 2.250mg Zn/l for 7 and 15 days, the ovaries showed separation and degeneration of the ovarian wall (Fig. 2f), dilation of the ovarian wall blood vessels (Fig. 2g), degenerative and necrotic changes in the oocytes (Figs. 2h,i), focal areas of necrosis in the ovarian stroma, haemorrhage between the oocytes (Fig. 2j), proliferation in the granulosa layer of oocytes (Fig. 2k,l) and separation of the follicular layers from the oocytes (Fig. 2m). Oocytes were atrophied and became abnormally irregular in shape (Fig. 2n). Also, yolk granules of many ova disappeared and the oocytes appeared empty (Fig. 2o).

**Ovaries of Fish Exposed to 0.725mg/l Cd:** The ovaries of fish exposed to 0.725mg Cd/l for 7 and 15 days showed separation and degeneration of the ovarian wall, degeneration and necrosis of the oocytes (Figs. 3a,b), focal areas of necrosis in the ovarian stroma (Fig. 3c), separation of the follicular layers from the oocytes

(Figs. 3d,e) as well as lysis of the yolk granules of mature oocytes (Fig. 3f). Intravascular haemolysis and dilation were seen in the ovarian wall blood vessels (Fig. 3g). Atrophy with distorted appearance was noticed in the oocytes (Figs. 3h-j). Moreover, after 15 days of exposure, coagulative necrosis in the yolk granules (Fig. 3k), liquification of the cytoplasm of mature oocytes (Figs. 3l,m) as well as dilation in the ovarian blood vessels (Fig. 3n) were observed.

**Ovaries of Fish Exposed to 1.450mg/l Cd:** The ovaries of fish exposed to 1.450mg Cd/l for 7 and 15 days showed severe degenerative changes in the oocytes (Figs. 3o,4a,b) with necrosis in the ovarian stroma, separation of the follicular layers from the oocytes (Figs. 4c,d), haemorrhage between the oocytes (Fig. 4e), intravascular haemolysis and dilation in the ovarian wall blood vessels (Fig. 4f), hyperplasia in the granulosa layer of the oocytes (Figs. 4g,h) and separation with degeneration of the ovarian wall (Fig. 4i). Oocytes collapsed and became abnormally irregular in shape (Figs. 4j,k). Lysis of the yolk granules of oocytes (Fig. 4l) and liquification of the cytoplasm of oocytes (Fig.4m) were noticed. Vitellogenic fluid was observed as a pink dark fluid filling the interstitial space or the ovarian parenchyma (Fig. 4n). Degeneration of zona radiata of the oocytes was noticed (Fig. 4o).

**Ovaries of Fish Exposed to the Mixture of Zn (1.125mg/l) and Cd (0.725mg/l):** After 7 and 15 days of exposure to the mixture of Zn (1.125mg/l) and Cd (0.725mg/l), the ovaries revealed severe degenerative and necrotic changes in the oocytes (Figs.5a-c), shrinkage with distorted appearance of oocytes (Figs. 5d,e), separation of the follicular layers from the oocytes and dilation in the ovarian wall blood vessels. In some atretic oocytes, their outer most layer of cytoplasm became liquefied. Lysis of the yolk granules of mature oocytes (Fig. 5f) was observed. In some mature oocytes, coagulative necrosis was noticed in the yolk granules (Figs. 5g,h). Vitellogenic fluid was observed in the ovarian cavity (Fig. 5i). Moreover, after 15 days of exposure, thickening of the ovarian wall (Fig. 5j) and hypertrophy with degeneration of zona radiata of the oocytes (Figs. 5k-m) were observed. Edema was noticed in some mature oocytes (Figs. 5n,o).

**Ovaries of Fish Exposed to the Mixture of Zn (2.250mg/l) and Cd (1.450mg/l):** The ovaries of fish exposed to the mixture of Zn (2.250mg/l) and Cd (1.450mg/l) for 7 and 15days showed separation of the ovarian wall (Fig. 6a), dilation in the ovarian wall blood vessels with thickening of the blood vessels wall (Fig. 6b), severe degeneration and necrosis in the oocytes (Figs. 6c-f), focal areas of necrosis in the ovarian stroma (Fig. 6g), atrophy with distorted appearance of oocytes (Fig. 6h,i), lysis of the yolk granules of mature oocytes (Fig. 6j) and intravascular haemolysis and dilation in the ovarian blood vessels (Fig. 6k). Liquification of the cytoplasm of oocytes and vitellogenic fluid in the ovarian cavity were seen.

**Histopathological Alterations in the Testes:**
**Control Testes:** The testes of fish from the control group showed normal histological structures (Figs. 7a).

**Testes of Fish Exposed to 1.125mg/l Zn:** The testes of fish exposed to 1.125mg Zn/l for 7 and 15days showed hypertrophy with vacuolar degeneration in spermatogonia (Figs.7b,c), degenerative and necrotic changes in the cellular elements of the seminiferous tubules with focal areas of necrosis (Figs.7d,e) and degeneration in the wall of the seminiferous tubules (Fig.7f). Sperms were scattered in the testis (Fig.7g). Seminiferous tubules appeared with a lesser number of sperms or empty (Figs.7d,h). Furthermore, after 15 days of exposure, degenerative changes were seen in the interstitial cells (Fig.7i).

**Testes of Fish Exposed to 2.250mg/l Zn:** Alterations exhibited in the testes of fish exposed to 2.250mg Zn/l for 7 and 15days were degenerative and necrotic changes in the cellular elements of the seminiferous tubules (Figs.7j-l), malformation and distortion in the architecture of seminiferous tubules (Fig.7m), degeneration in the wall of seminiferous tubules as well as in the interstitial cells (Fig. 7m) and haemorrhage between the seminiferous tubules (Fig.7n). Furthermore, seminiferous tubules appeared with a lesser number of sperms (Fig. 7o). The severity of lesions was progressed with the progression of the experimental period.

**Testes of Fish Exposed to 0.725mg/l Cd:** After 7 and 15 days of exposure to 0.725mg Cd/l, the testes revealed degeneration of the cellular elements of the seminiferous tubules with necrotic focal areas (Figs. 8a,b) and degeneration in the wall of seminiferous tubules as well as in the interstitial cells (Fig. 8c-e). The overall structure of the testis looked disrupted. Moreover, after 15 days of exposure, atrophy was noticed in the seminiferous tubules (Fig. 8f) with reduction in the size of seminiferous tubules lumen (Fig. 8f).

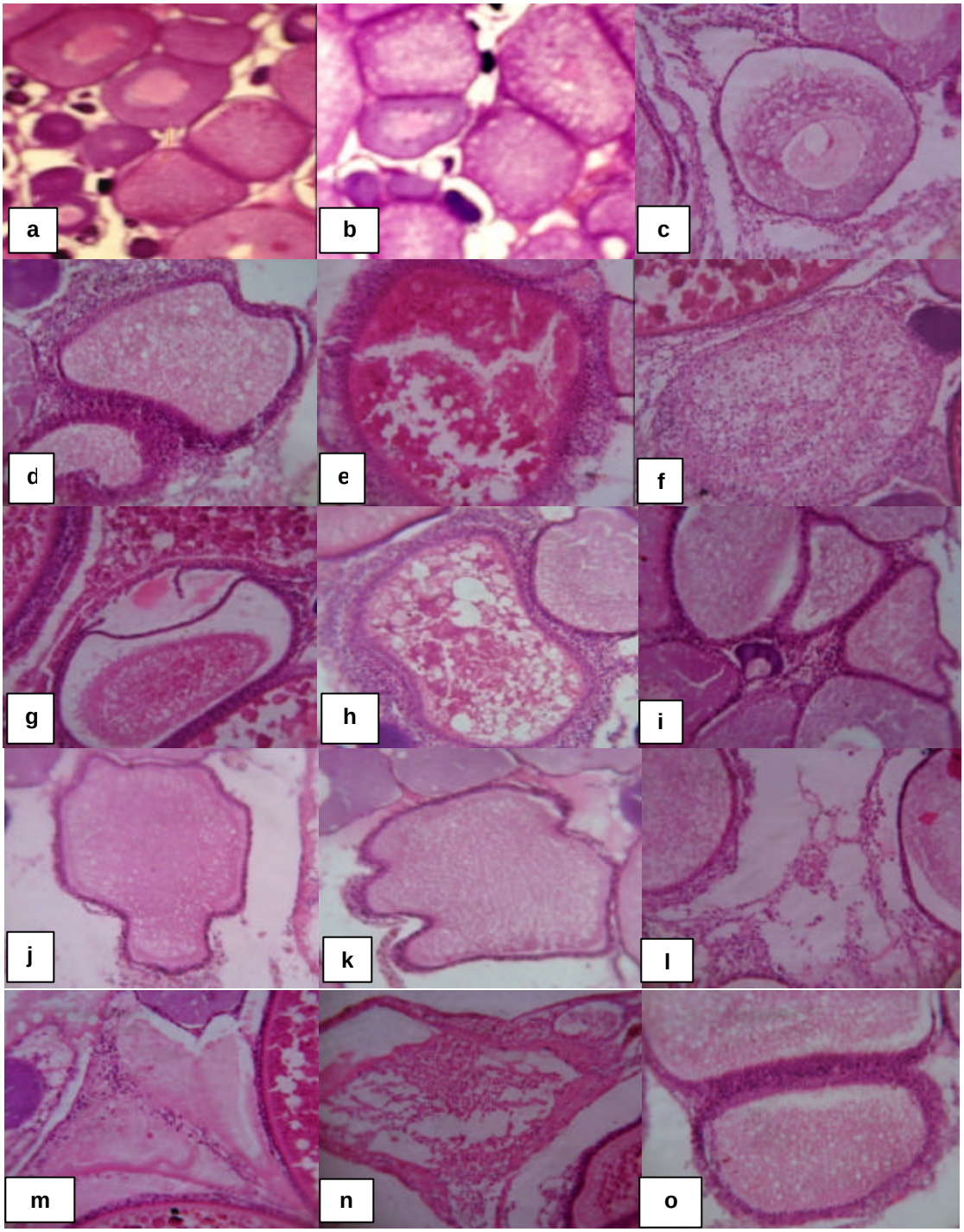


Fig. 1: Ovary of fish showing the control (a,b)(X250), degenerative and necrotic changes (atresia) in oocytes (c,d,e,f,g,h [1.125mg Zn/l-7&15days])(X400), shrinkage with distorted appearance of oocytes (i,j,k [1.125mg Zn/l-7days])(X400), focal area of necrosis in the ovarian stroma (l [1.125mg Zn/l-15days])(X400), haemorrhage between the oocytes (m [1.125mg Zn/l-15days])(X400), dilation of ovarian wall blood vessel (n [1.125mg Zn/l-7days])(X400), proliferation in the granulosa layer of oocytes (o [1.125mg Zn/l-7days])(X400).

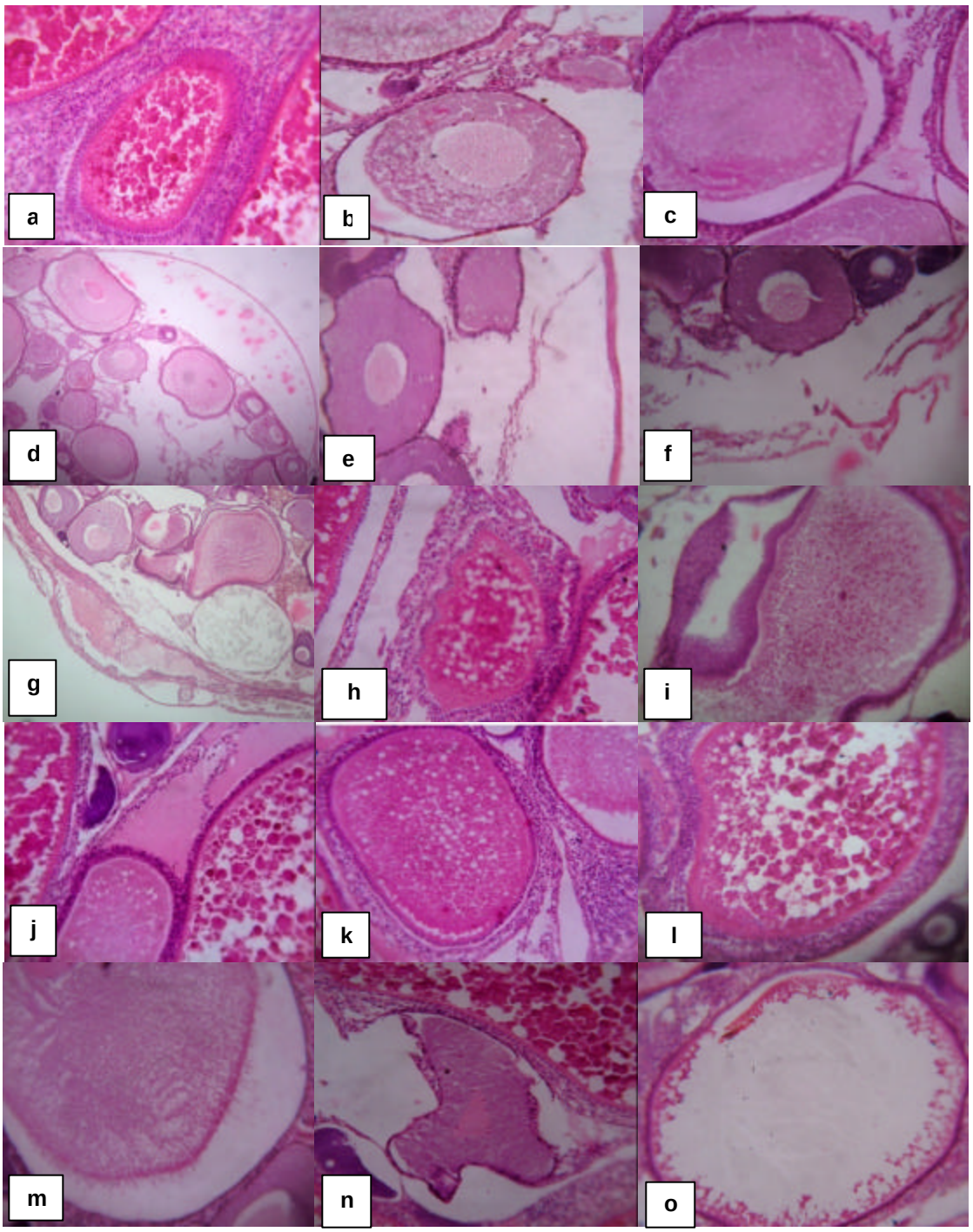


Fig. 2: Ovary of fish showing proliferation in the granulosa layer of oocytes (a [1.125mg Zn/l-15days])(X400), separation of the follicular layers from the oocytes (b,c [1.125mg Zn/l-15days])(X400), separation of the ovarian wall (d,e [1.125mg Zn/l-7days])(X250&400,respectively), separation and degeneration of the ovarian wall (f [2.250mg Zn/l-7days])(X400), dilation of ovarian wall blood vessel (g [2.250mg Zn/l-15days])(X250), degenerative and necrotic changes in the oocytes (h,i [2.250mg Zn/l-15days])(X400), haemorrhage between the oocytes (j [2.250mg Zn/l-7days])(X400), proliferation in the granulosa layer of oocytes (k,l [2.250mg Zn/l-7days])(X400), separation of the follicular layers from the oocyte (m [2.250mg Zn/l-7days])(X400), atrophy in the oocyte (n [2.250mg Zn/l-15days])(X400), disappearance of yolk granules of the oocyte (o [2.250mg Zn/l-15days])(X400)

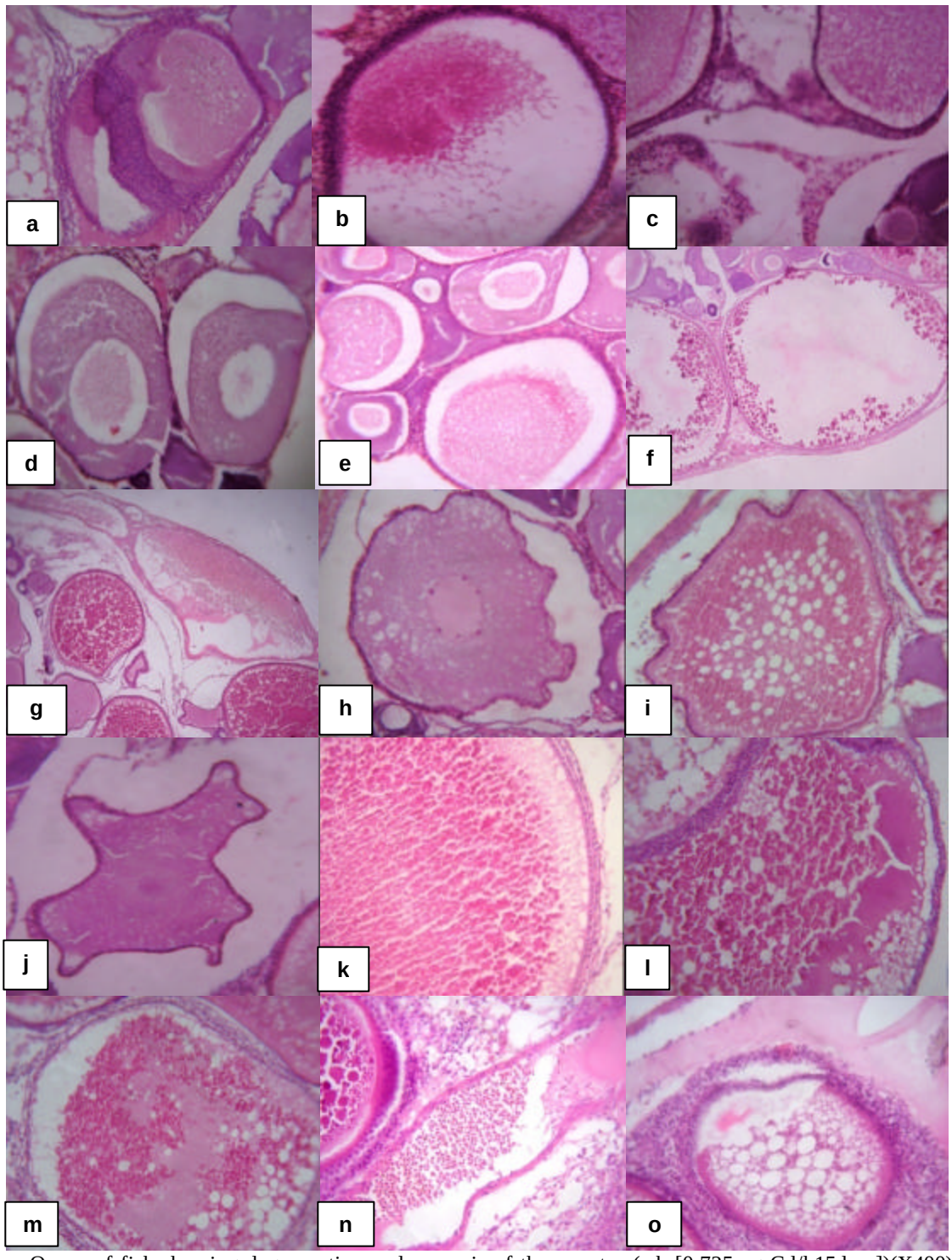


Fig. 3: Ovary of fish showing degeneration and necrosis of the oocytes (a,b [0.725mg Cd/l-15days])(X400), focal area of necrosis in ovarian stroma (c [0.725mg Cd/l-7days])(X400), separation of the follicular layers from the oocytes (d,e [0.725mg Cd/l-7days])(X400), lysis in the yolk granules of the oocyte (f [0.725mg Cd/l-15days])(X400), intravascular haemolysis and dilation in ovarian wall blood vessel (g [0.725mg Cd/l-7days])(X250), atrophy in the oocytes (h,i,j [0.725mg Cd/l-15days])(X400), coagulative necrosis in the yolk granules (k [0.725mg Cd/l-15days])(X400), liquification of the cytoplasm of mature oocytes (l,m [0.725mg Cd/l-15days])(X400), dilation in ovarian blood vessel (n [0.725mg Cd/l-15days])(X400), degenerative changes in the oocyte (o [1.450mg Cd/l-7days])(X400)

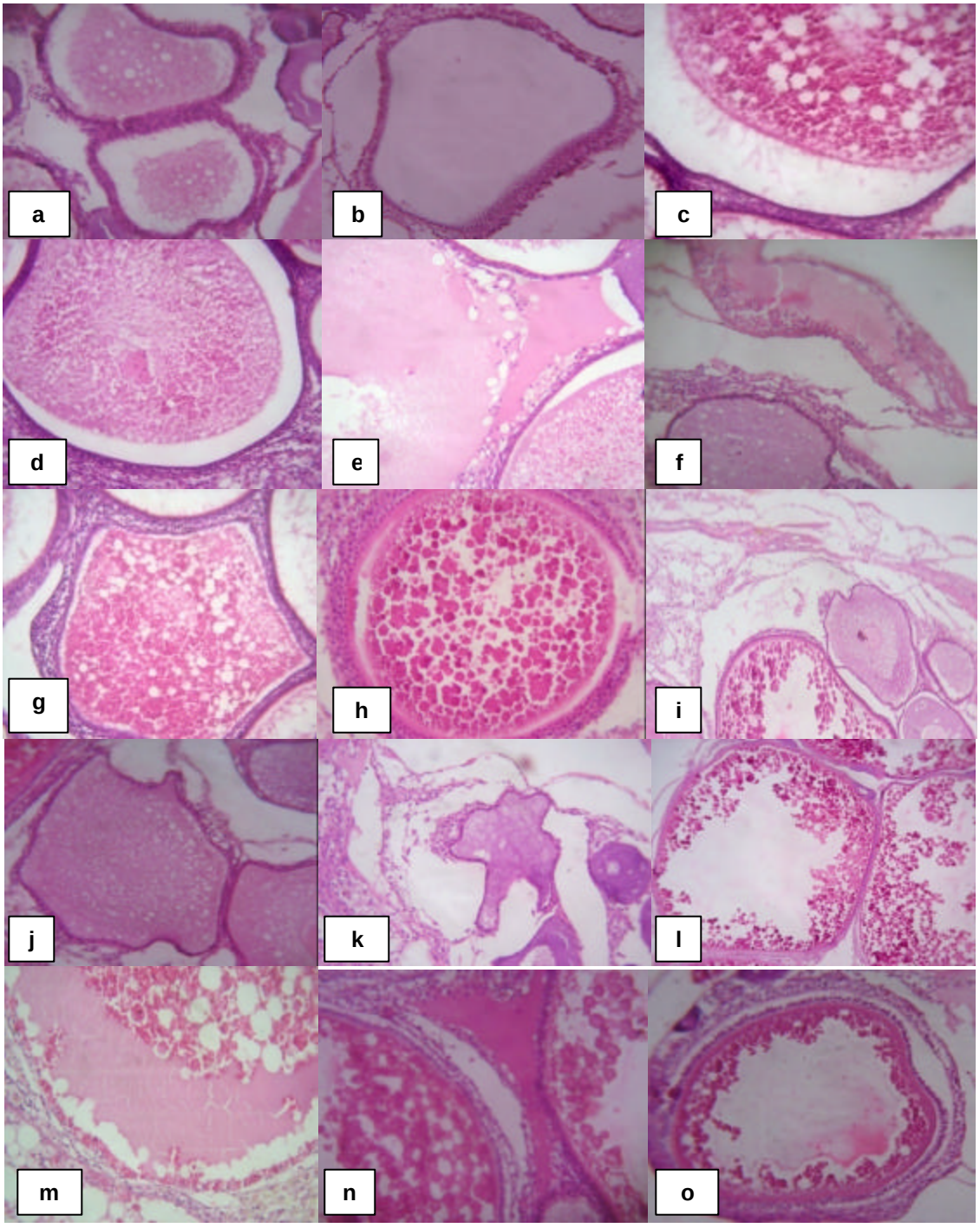


Fig. 4: Ovary of fish showing severe degenerative changes in the oocyte (a,b [1.450mg Cd/l-15days])(X400), separation of the follicular layers from the oocytes (c,d [1.450mg Cd/l-7&15days])(X400),haemorrhage between the oocytes (e [1.450mg Cd/l-15days])(X400), intravascular haemolysis and dilation in ovarian wall blood vessel (f [1.450mg Cd/l-7days])(X400), proliferation in the granulosa layer of oocytes (g,h [1.450mg Cd/l-7&15days])(X400), separation and degeneration of the ovarian wall (i [1.450mg Cd/l-7days])(X400), collapse in the oocytes (j,k [1.450mg Cd/l-7&15days])(X400), lysis in the yolk granules of the oocytes (l [1.450mg Cd/l-15days])(X400), liquification of the cytoplasm of mature oocyte (m [1.450mg Cd/l-7days])(X400), vitellogenic fluid in the ovarian cavity (n [1.450mg Cd/l-15days])(X400), degeneration of zona radiata of the oocyte (o [1.450mg Cd/l-7days])(X400)

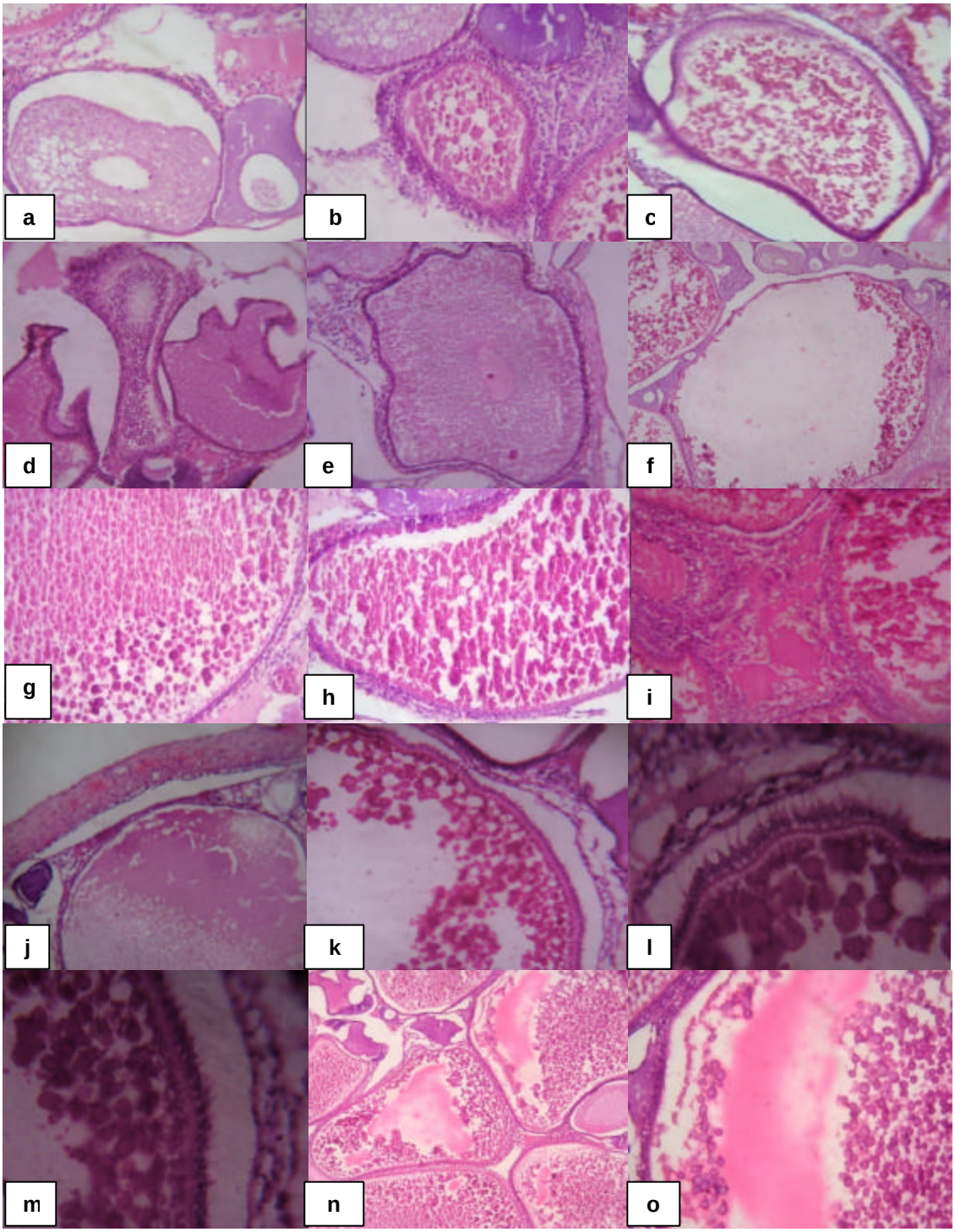


Fig. 5: Ovary of fish showing severe degenerative and necrotic changes in the oocyte (a,b,c [1.125mg Zn/l and 0.725mg Cd/l-7&15days])(X400), shrinkage with distorted appearance of oocytes (d,e [1.125mg Zn/l and 0.725mg Cd/l-15days])(X400), lysis in the yolk granules of the oocytes (f [1.125mg Zn/l and 0.725mg Cd/l-7days])(X400), coagulative necrosis in the yolk granules (g,h [1.125mg Zn/l and 0.725mg Cd/l-15days])(X400), vitellogenic fluid in the ovarian cavity (i [1.125mg Zn/l and 0.725mg Cd/l-7days])(X400), thickening of the ovarian wall (j [1.125mg Zn/l and 0.725mg Cd/l-15days])(X400), hypertrophy with degeneration of zona radiata of the oocyte (k,l,m [1.125mg Zn/l and 0.725mg Cd/l-15days])(X400&1000,respectively), edema in mature oocytes (n,o [1.125mg Zn/l and 0.725mg Cd/l-15days])(X250&400, respectively).

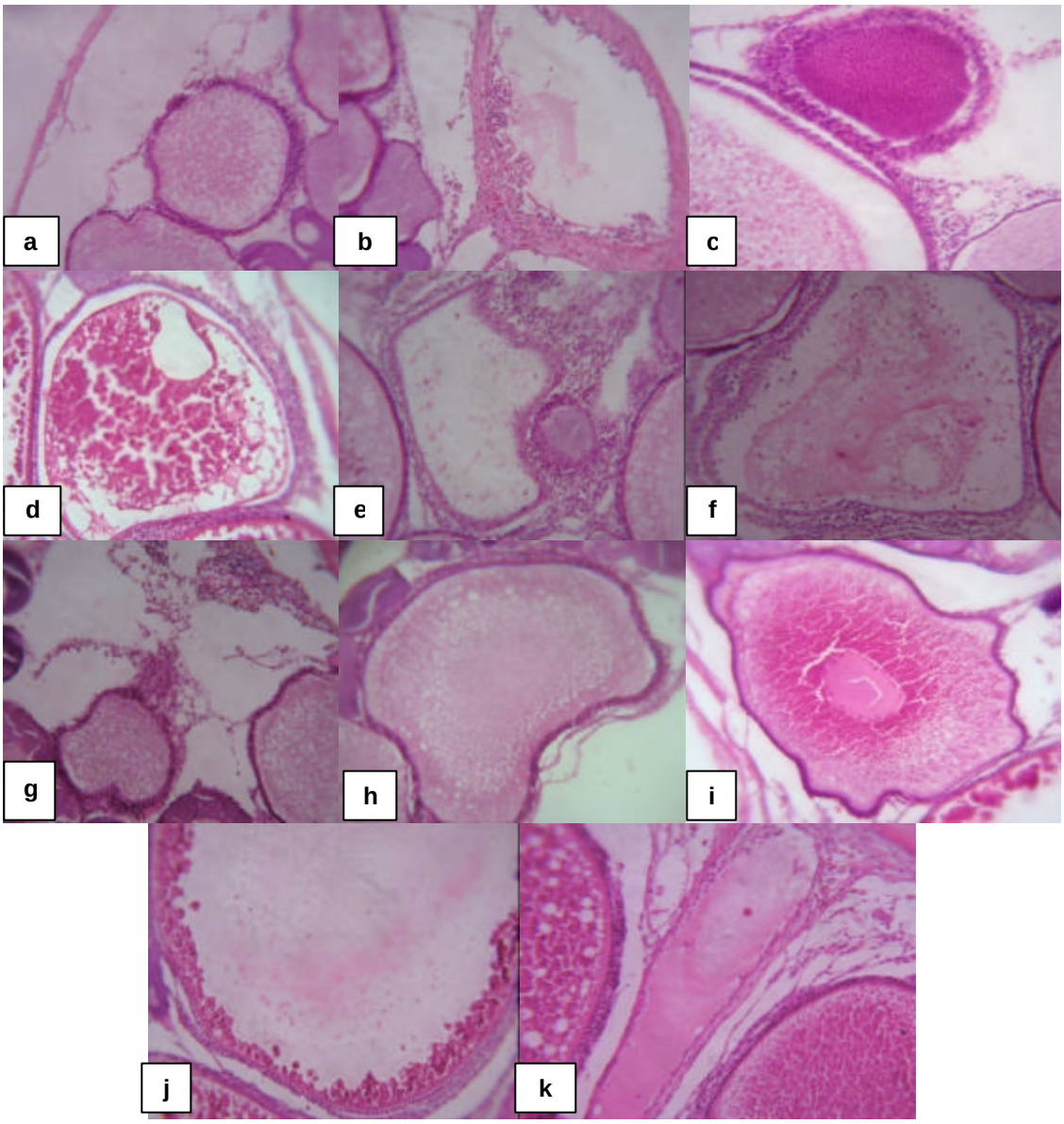


Fig. 6: Ovary of fish showing separation of the ovarian wall (a [2.250mg Zn/l and 1.450mg Cd/l-7days])(X400), dilation in ovarian wall blood vessel (b [2.250mg Zn/l and 1.450mg Cd/l-7days])(X400), severe degeneration and necrosis in the oocytes (c,d,e,f [2.250mg Zn/l and 1.450mg Cd/l-7&15days])(X400), focal area of necrosis in the ovarian stroma (g [2.250mg Zn/l and 1.450mg Cd/l-15days])(X400), atrophy with distorted appearance of oocytes (h,i [2.250mg Zn/l and 1.450mg Cd/l-7days])(X400), lysis in the yolk granules of the oocyte (j [2.250mg Zn/l and 1.450mg Cd/l-15days])(X400), intravascular haemolysis and dilation in ovarian blood vessel (k [2.250mg Zn/l and 1.450mg Cd/l-15days])(X400).

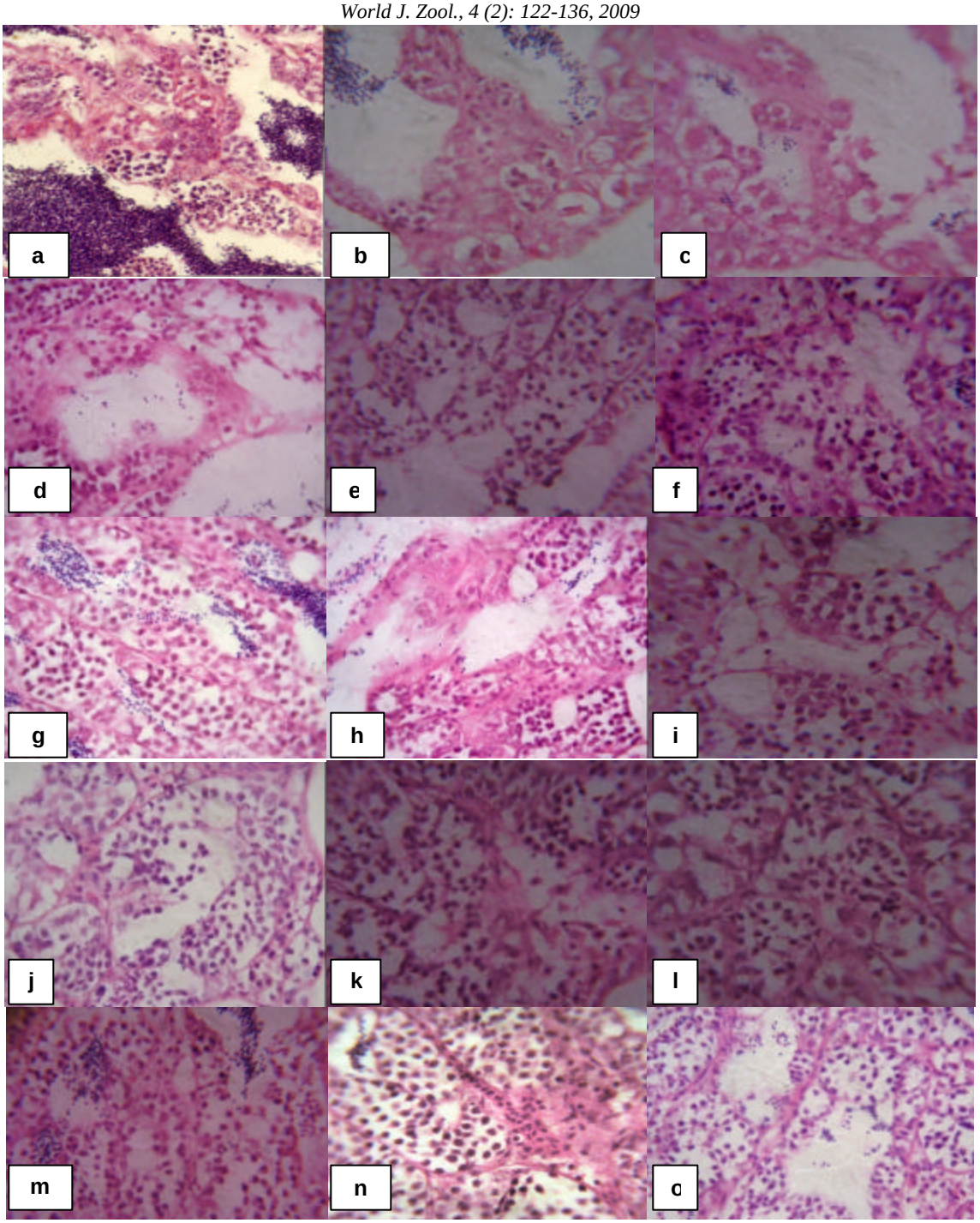


Fig. 7: Testis of fish showing the control (a)(X400), hypertrophy with vacuolar degeneration in spermatogonia (b,c [1.125mg Zn/l-15days])(X1000), degenerative and necrotic changes in the cellular elements of the seminiferous tubules with focal areas of necrosis (d,e [1.125mg Zn/l-7&15days, respectively])(X400), degeneration in the wall of seminiferous tubules (f [1.125mg Zn/l-7days])(X400), sperms scattered in the testis (g [1.125mg Zn/l-7days])(X400), seminiferous tubules with a lesser number of sperms (h [1.125mg Zn/l-7days])(X400), degenerative changes in the interstitial cells (i [1.125mg Zn/l-15days])(X400), degenerative and necrotic changes in the cellular elements of the seminiferous tubules (j,k,l [2.250mg Zn/l-7&15days, respectively])(X400), malformation and distortion in the architecture of seminiferous tubules and degeneration in the wall of seminiferous tubules as well as in the interstitial cells (m [2.250mg Zn/l-15days])(X400), haemorrhage between the seminiferous tubules (n [2.250mg Zn/l-7days])(X400), seminiferous tubules with a lesser number of sperms (o [2.250mg Zn/l-7days])(X400).

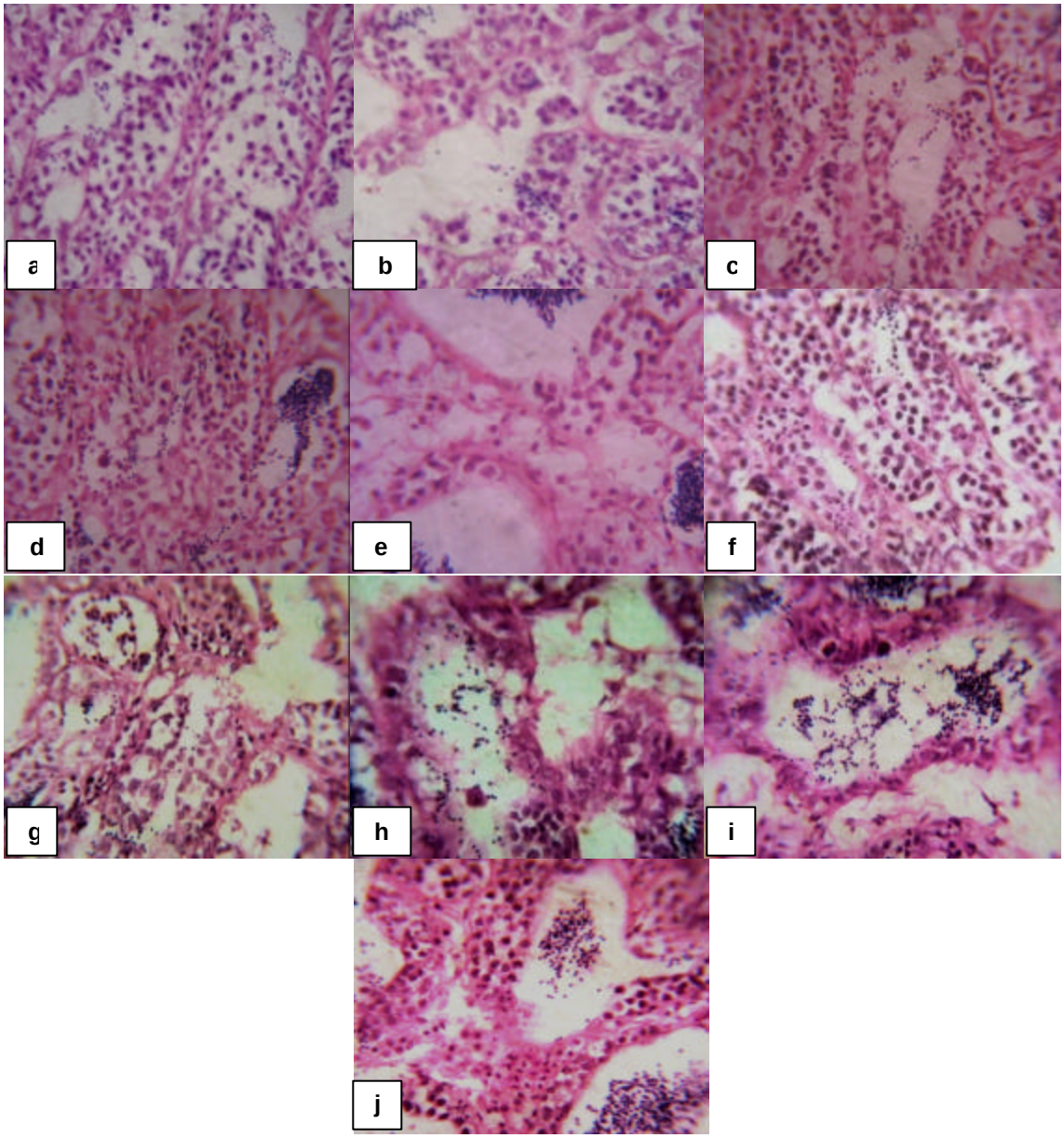


Fig. 8: Testis of fish showing degeneration of the cellular elements of the seminiferous tubules with necrotic focal areas (a,b [0.725mg Cd/l-7days])(X400), degeneration in the wall of seminiferous tubules as well as in the interstitial cells (c,d,e [0.725mg Cd/l-15days])(X400&1000, respectively), atrophy in the seminiferous tubules with reduction in the size of seminiferous tubules lumen (f [0.725mg Cd/l-15days])(X400), malformation and distortion in the architecture of seminiferous tubules with severe degenerative and necrotic changes in the cellular elements of the seminiferous tubules, focal areas of necrosis and degeneration in the wall of seminiferous tubules as well as in the interstitial cells (g,h [1.450mg Cd/l-15days])(X400), focal areas of necrosis amongst the sperms (i [1.450mg Cd/l-15days])(X400), haemorrhage between the seminiferous tubules (j [1.450mg Cd/l-7days])(X400).

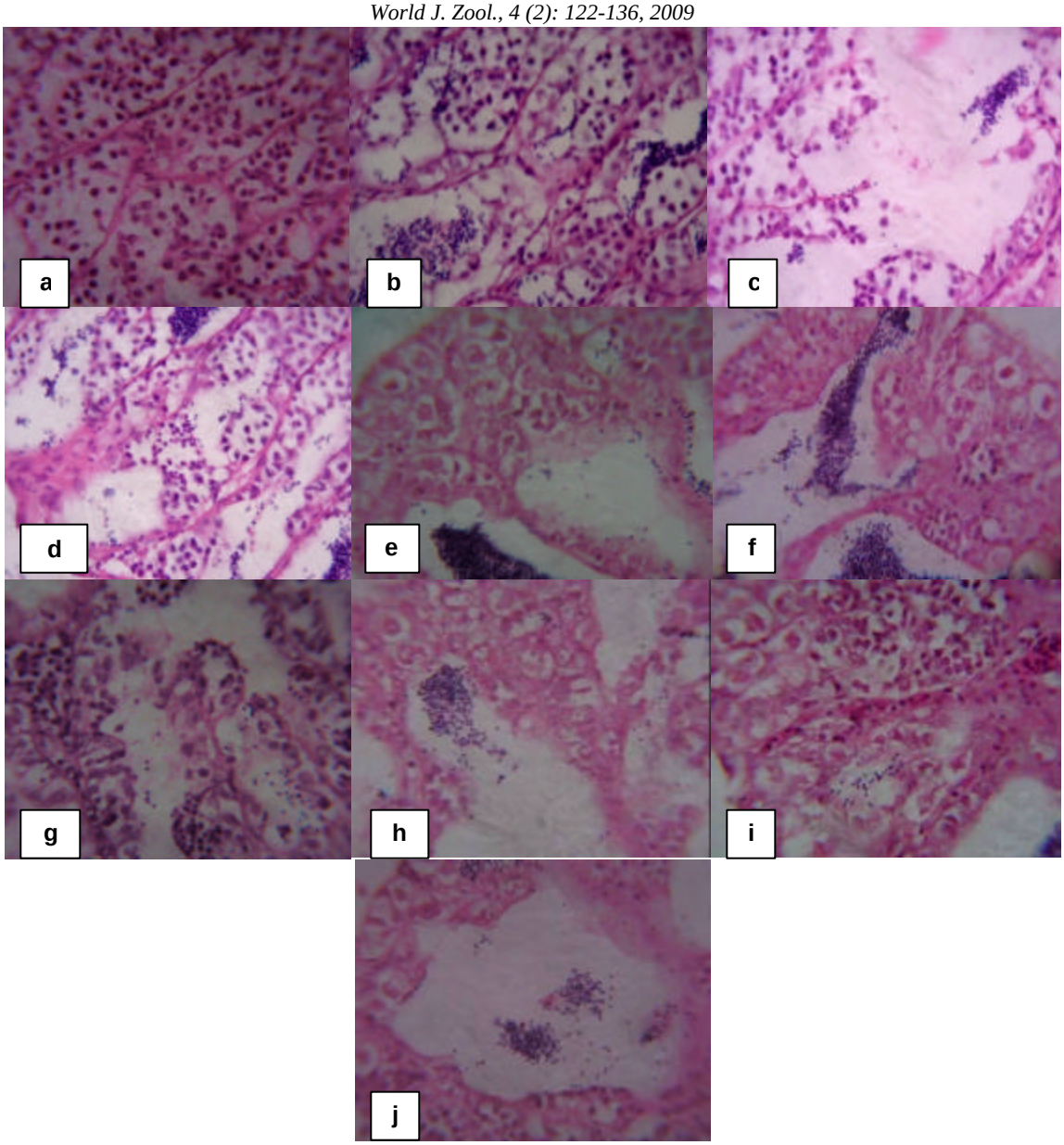


Fig. 9: Testis of fish showing severe degeneration and necrosis in the cellular elements of the seminiferous tubules (a,b,c [1.125mg Zn/l and 0.725mg Cd/l-7&15days, respectively])(X400), seminiferous tubules with a lesser number of sperms (d [1.125mg Zn/l and 0.725mg Cd/l-7days])(X400), hypertrophy with vacuolar degeneration in spermatogonia (e,f [2.250mg Zn/l and 1.450mg Cd/l-7days])(X400), severe degenerative and necrotic changes in the cellular elements of the seminiferous tubules (g,h [2.250mg Zn/l and 1.450mg Cd/l-7&15days, respectively])(X400), haemorrhage between the seminiferous tubules (i [2.250mg Zn/l and 1.450mg Cd/l-7days])(X400), seminiferous tubules with a lesser number of sperms (j [2.250mg Zn/l and 1.450mg Cd/l-15days])(X400).

**Testes of Fish Exposed to 1.450mg/l Cd:** The testes of fish exposed to 1.450mg Cd/l for 7 and 15days showed malformation and distortion in the architecture of the seminiferous tubules (Figs. 8g,h) with severe degenerative and necrotic changes in the cellular elements of the seminiferous tubules, focal areas of necrosis and degeneration in the wall of seminiferous tubules as well as in the interstitial cells (Figs. 8g,h). Focal areas of necrosis were noticed amongst the sperms (Fig.8i). Moreover, haemorrhage was seen between the seminiferous tubules (Fig. 8j).

**Testes of Fish Exposed to the Mixture of Zn (1.125mg/l) and Cd (0.725mg/l):** The testes of fish exposed to the mixture of Zn (1.125mg/l) and Cd (0.725mg/l) for 7 and 15days showed atrophy in the seminiferous tubules, severe degeneration and necrosis in the cellular elements of seminiferous tubules (Figs.9a-c), focal areas of necrosis (Fig.9c) and degeneration in the wall of seminiferous tubules (Fig. 9c) as well as in the interstitial cells. Many seminiferous tubules appeared with a lesser number of sperms (Fig. 9d). Focal areas of necrosis were seen amongst the sperms.

**Testes of Fish Exposed to the Mixture of Zn (2.250mg/l) and Cd (1.450mg/l):** After 7 and 15days of exposure to the mixture of Zn (2.250mg/l) and Cd (1.450mg/l), the testes revealed hypertrophy with vacuolar degeneration in spermatogonia (Figs. 9e,f), severe degenerative and necrotic changes in the cellular elements of the seminiferous tubules (Figs.9g,h), focal areas of necrosis (Fig. 9h), degeneration in the wall of seminiferous tubules as well as in the interstitial cells and haemorrhage between the seminiferous tubules (Fig. 9i). Seminiferous tubules appeared with a lesser number of sperms (Fig. 9j).

## DISCUSSION

The results of this work clearly indicated that zinc and/or cadmium had adverse effects on the ovaries and testes of *O. niloticus*. The severity of lesions caused by Zn and/or Cd was correlated with the concentration and duration of exposure. Also, the results indicated that Cd is more toxic to the studied fish than Zn.

This study showed that Zn and/or Cd exposure cause separation and degeneration of the ovarian wall, degenerative and necrotic changes (atresia) in oocytes, shrinkage with distorted appearance of oocytes, separation of the follicular layers from oocytes, proliferation in the granulosa layer of oocytes, lysis of yolk granules, liquification of the cytoplasm of oocytes as well as focal areas of necrosis in the ovarian stroma in the ovaries of *O. niloticus*. Intravascular haemolysis and dilation in the ovarian blood vessels were also seen. Furthermore, vitellogenic fluid was noticed in the ovarian cavity. The results of this study are similar to those recorded in other fish exposed to different heavy metals [22-25]. Also, Kumar and Pant [12] reported a significant atresia in the ovary with major damage to younger oocytes in *Puntius conchonius* exposed to zinc, they suggested a direct action of zinc on the ovaries. Hanna *et al.* [15] showed that the number of mature ova was decreased, with high incidence of atresia in the ovaries of *O. niloticus* subjected to lead and chromium. Moreover, they noticed that the mature ova was empty or contained large number of fat granules.

Wahbi and El-Greisy [26] observed extensive necrosis of oolema, atresia in the mature follicles, broken zona radiata, proliferation of follicular cells of oocytes and break down of yolk granules in the ovaries of *Siganus rivulatus* after exposure to different waste sources (containing Zn). In the ovaries of *Clarias gariepinus* exposed to lead, diffuse mild degeneration and necrosis of the follicles were initially observed, subsequently, marked severe degeneration of ovarian follicles were seen [18].The results of Mishra and Mohanty [19] showed that exposure to chromium caused increase in the percentage of atretic oocytes and decrease in the percentage of vitellogenic oocytes in the ovaries of *Channa punctatus*. Furthermore, Verma and Srivastava [20] observed shrinkage of oocytes, which resulted in large interfollicular spaces, distorted appearance of oocytes and increase in number of atretic follicles in the ovaries *Channa punctata* exposed to zinc.

Ovarian changes noted in the present study can be attributed to direct action of Zn and Cd on the ovary [12,20] as well as due to indirect effect on the higher centre with consequent inhibitory action on the pituitary-gonadal axis [27,28]. Also, the observed alterations may be mediated by overall systemic toxicity affecting other vital organs [19].

In the present investigation, Zn and Cd are notable as testicular toxicants because the testes were highly affected by both metals. Zn and/or Cd induced hypertrophy with vacuolar degeneration in spermatogonia, degenerative and necrotic changes in the cellular elements of the seminiferous tubules, focal areas of necrosis, degeneration in the wall of seminiferous tubules as well as in the interstitial cells and atrophy in

the seminiferous tubules. The overall structure of the testes looked disrupted and the seminiferous tubules appeared with a lesser number of sperms or empty. These results were in agreement with those observed by many investigators who have investigated the effects of different heavy metals on fish testes. Kumar and Pant [12] reported that zinc exposure caused inhibition in spermatogenesis in the testes of *Puntius conchonius*. Kumari and Dutt [29] observed disorganization in the testicular tubules in the testes of *Puntius sarana* exposed to cadmium. Kirubagaran and Joy [14] observed progressive degenerative changes in the interstitial Leydig cells and reduction in the size of seminiferous tubules in the testes of *Clarias batrachus* exposed to mercury. Moreover, Hanna *et al.* [15] reported that lead and chromium caused atrophy in the seminiferous tubules, vacuolation and necrosis of the spermatocytes, a decrease in numbers of sperms in the lumen of seminiferous tubules as well as vacuolation and necrosis of the germinal epithelium of testes of *O. niloticus*. Yamaguchi *et al.* [17] showed that lead, molybdenum, rubidium and arsenic induced necrosis of spermatogonia and might inhibit spermatogenesis in Japanese eel.

As the spermatogonia are the initial sites of spermatogenesis, their hypertrophy and degeneration observed in this study may hinder the production of viable spermatozoa, endangering the population dynamics.

The interstitial cells which are steroidogenic in the testes of several teleosts including *O. niloticus* [30] showed degenerative changes. These cellular alterations reflect adversely on the steroidogenic potential of the cells. The resulting impaired steroidogenic activity might have led to the arrest of spermatogenic activity [14]. An impairment of steroidogenesis has been reported after treatment with cadmium in *Salvelinus fontinalis* [31] and *Oncorhynchus mykiss* [32] testes.

The histopathological alterations observed in the ovaries and testes of *O. niloticus* indicated that Zn and Cd represent critical stress for reproduction. Both metals inhibited the process of spermatogenesis and altered the developing eggs within the fish testes and ovaries, respectively and ultimately reduce the potential numbers available at spawning.

In conclusion, the results of this study showed that Zn and/or Cd exposure at the concentrations and durations mentioned caused many histopathological alterations in the gonads (ovaries and testes) of *O. niloticus* which can lead to reduction in fertility and fish population.

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