# Chapter 4

# Results and Discussion

## 4.1 Acute toxicity of neem *A. indica* seed extract on Nile tilapia *O. niloticus*

From data obtained, the median lethal concentration ($LC_{50}$) of neem seed extract to *O. niloticus* at 96 hours of exposure was determined at 36.25 ppm. Time-related decrease in $LC_{50}$ values was presented. The $LC_{50}$ were 47.71, 40.71, 38.92 and 36.25 ppm at the exposure period of 24, 48, 72 and 96 hours, respectively (Table 3-3, Figure 4-1).

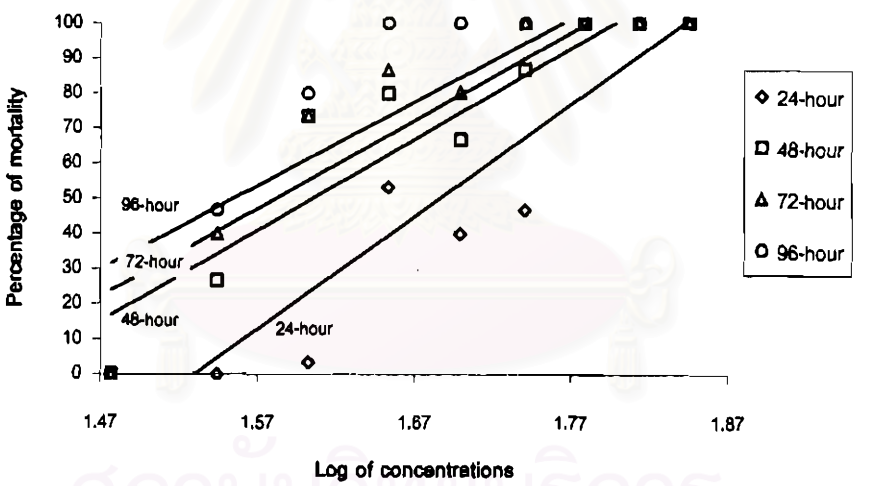



Figure 4-1 Percentage of mortality of Nile tilapia *O. niloticus* after different exposure time of acute toxicity test.

Different $LC_{50}$ values of various neem products were documented in previous studies. Larson (1989) cited in Jacobson (1995) reported the 96-h $LC_{50}$ of Margosan-O, a trade neem product, in water against rainbow trout at 8.8 ml/l. In semi-field trial against fingerlings of tilapia *O. niloticus*, the $LC_{50}$ of neem oil at 24 hours was determined in aquarium at 1124.6 ppm (Fernandez et al., 1992 cited in Jacobson, 1995). Osuala and Okwuosa (1993) reported the 96-h $LC_{50}$ of stem bark extract of neem against fish

*Aphyosemon giardneri* at 15.1 mg/l. Wan et al. (1996) also reported the 96-h $LC_{50}$ of neem extract against juvenile Pacific Northwest salmon at 7±3 mg/l. These different values are depended on species, formulation of neem products, duration time of exposure, test conditions and test assessment.

## 4.2 Subchronic toxicity of neem *A. indica* seed extract on Nile tilapia *O. niloticus*.

The sublethal concentration (AF) of the neem seed extract used in this study was calculated from the NOEC, LOEC and $LC_4$ value at 25.07 ppm. After subchronic exposure to this concentration for 7 months, the effects were observed in the 4th, 5th, 6th and 7th month of experiment comparing between control and treated group.

### 4.2.1 Gonadosomatic index (GSI)

Mean body weight, ovaries weight and GSI of control and treated fish belonging to different experimental period are shown in table 4-1.

Table 4-1 Mean body weight, ovaries weight and GSI for female *O.niloticus* of control and neem treated group in different experimental period. All values are shown in mean±SE.

| | Experimental groups | Experimental periods (month) | | | |
|---|---|---|---|---|---|
| | | 4th | 5th | 6th | 7th |
| Body Weight (g) | Control | 24.9±1.17 (n=19) | 32.41±1.95 (n=20) | 34.27±3.32 (n=21) | 39.95±2.19 (n=21) |
| | Treated | 16.87±1.02* (n=20) | 23.27±1.54* (n=19) | 28.01±2.04 (n=20) | 18.83±1.44* (n=21) |
| Ovaries Weight (g) | Control | 0.60±0.09 | 1.21±0.18 | 1.43±0.20 | 1.66±0.16 |
| | Treated | 0.29±0.06* | 0.75±0.13* | 0.79±0.13* | 0.71±0.07* |
| GSI | Control | 2.48±0.37 | 3.72±0.46 | 4.12±0.43 | 4.10±0.34 |
| | Treated | 1.66±0.37 | 3.07±0.44 | 2.63±0.36* | 3.96±0.35 |

* Indicate significant differences from respective controls ($p \leq 0.05$).

There were treatment-related effects on growth. Body weight of neem treated fish were significantly lower than that of control fish and were found to droped at the end of experiment. Ovaries weight of the treated fish were also presented in the same manner with significant differences in all experimental periods (Figure 4-2).

Female reproductive development was quantified by the GSI. From the data obtained, The GSI of treated fish in all treated periods were lower than that of controls, especially in the 6th month there was significant difference from control ($p \leq 0.05$). The GSI of control group increased in the initial phase of experiment (4th and 5th month) and became stable at the later experimental periods (6th and 7th month). The control GSI ranged from 2.48±0.37 at the beginning of sampling period to 4.10±0.34 at the end of the experiment. On the other hand, GSI of neem treated group ranged from 1.66±0.37 at the beginning of sampling period to 3.96±0.35 at the end of the experiment. The treated GSI droped significantly at the 6th month (Figure 4-3).

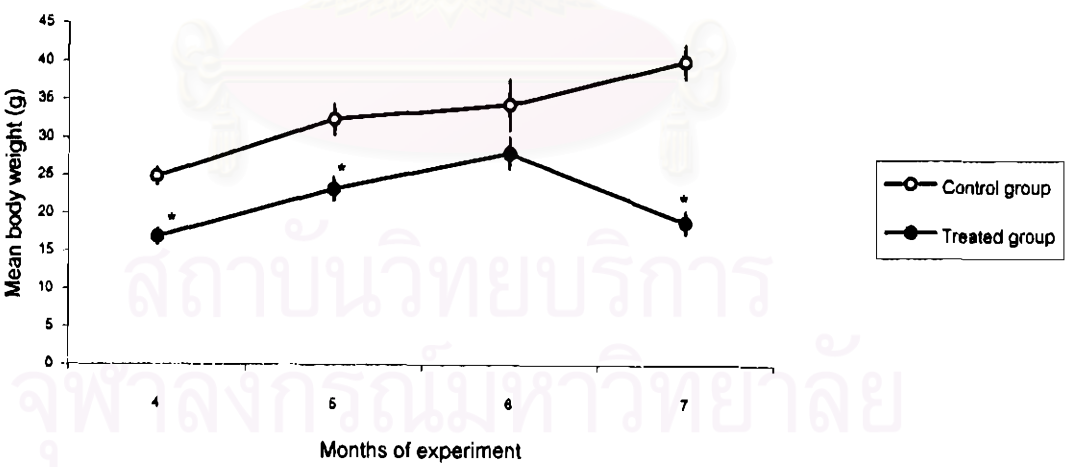



Figure 4-2 Mean (±SE) body weight of control and treated Nile tilapia after subchronic exposure to neem seed extract. *Indicate significant differences from respective controls ($p \leq 0.05$).

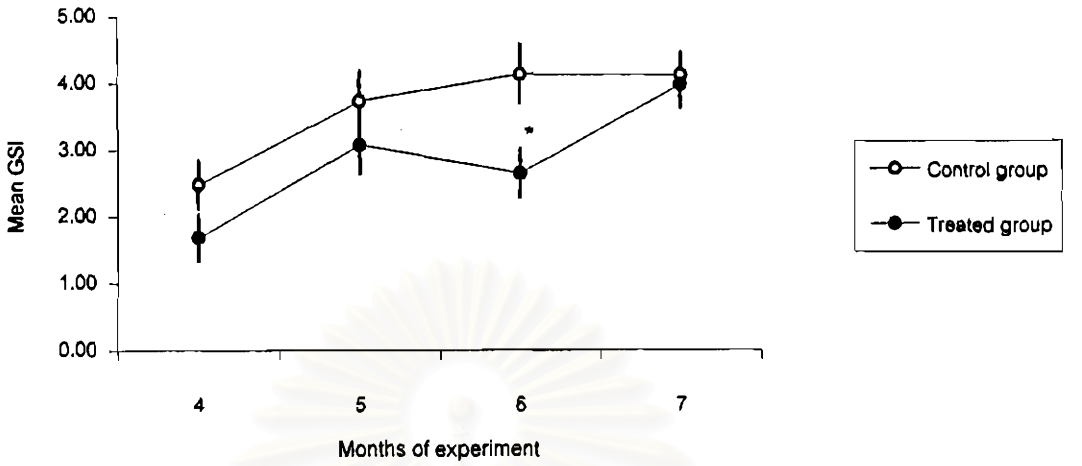



Figure 4-3 Mean (±SE) gonadosomatic indices (GSI) of control and treated tilapia after subchronic exposure to neem seed extract, *indicate significant differences from respective controls ($p \leq 0.05$).

In the three-spined stickleback, *Gasterosteus aculeatus* exposed to bis (tributyltin)oxide for 7.5 months in a flow-through system, it was also found that GSI in control fish increased significantly while that in the exposed groups remained unchanged (Holm et al., 1991). From a toxicity study of ammonium sulfate on *Channa punctatus*, the female GSI was also significantly reduced (Ram and Sathyanesan, 1986). In general, correlated with the results of the present study, the GSI of fertile (2n) female tilapia *O. niloticus* was gradually increased throughout the successive age of maturity between 4 to 10 months (Hussain et al., 1996). The lower GSI in the neem treated tilapia with significant decrease in the 6th month, therefore indicate reproductive impairment from the neem extract to the fish.

4.2.2 Fecundity

Fecundity of female tilapia of both experimental groups was determined by counting the number of eggs in each individual. Nile tilapia have asynchronous ovaries,

in which oocytes at all stages of development are presented. Therefore, the ovary contained many mode of oocyte size.

The oocytes were separately counted in 3 size groups depending on egg diameter and external characteristics. Thirty oocytes were sampled from the ovary of each experimental group and were measured for their longest dimension of diameter (horizontal diameter). There were significant differences ($p \leq 0.05$) of the diameter among groups of size separated as shown in figure 4-4.

The largest, ripe oocytes were deep-yellow to orange in color and ovoid in shape. Mean horizontal diameter was 1.76±0.04 mm (n=80) (Figure 4-5).

The intermediate, yolked oocytes were yellow to pale yellow in color and spherical in shape with mean horizontal diameter of 0.77±0.01 mm (n=80) (Figure 4-5).

The small, immature oocytes were pale yellow to white in color or transparent and spherical in shape. The mean horizontal diameter was 0.22±0.01 mm (n=80). This size group was not counted because of their very small size. Their number were calculated from dry weight of the ovarian tissue left after the large and intermediate oocytes were separated. The number of oocytes in each group is shown in appendix III.

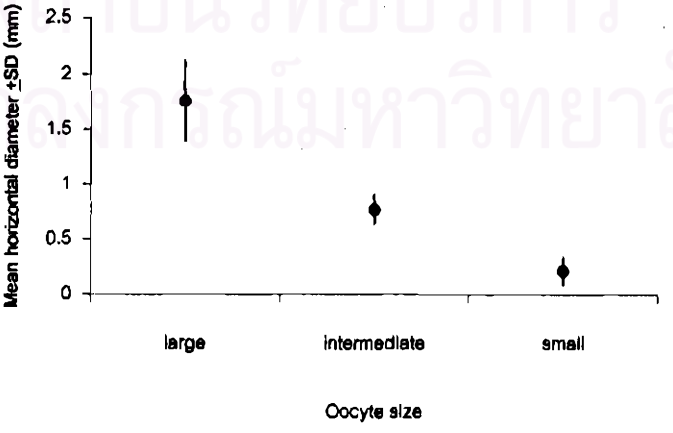



Figure 4-4 Mean (±SD) horizontal diameter of Nile tilapia oocytes in each size group.

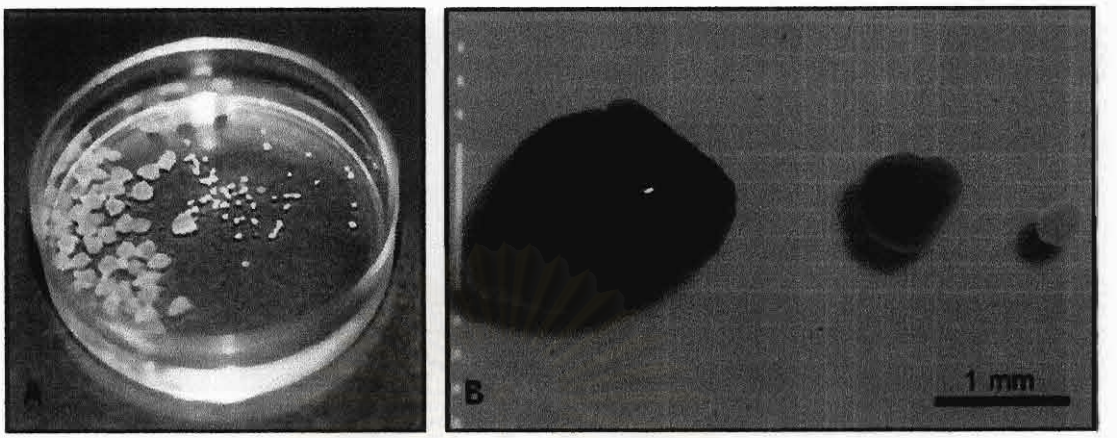



Figure 4-5 A. Macroscopic appearance of the oocytes during counting process. B. Stereomicroscopic appearances of the large, intermediate and small oocyte.

The total number of oocytes in the ovary of treated group were significantly different from that of control group in 4th and 7th month as shown in figure 4-6.

The number of the largest, ripe oocytes implied the number of eggs spawned. Therefore, fecundity was well determined by counting the number of ripe oocytes. There were significant differences between control and treated group in all experimental period as presented in figure 4-7.

The number of intermediate oocytes indicated significant differences between control and treated group from the 6th month until the end of experiment. The mean number of intermediate oocytes of treated group were lower than that of controls in all experimental periods (Table 4-2).

The number of small oocytes showed significant differences between control and treated group at the 4th and the 7th month of experiment. The mean number of the oocytes of treated group were also lower than that of controls in all experimental periods (Table 4-2).

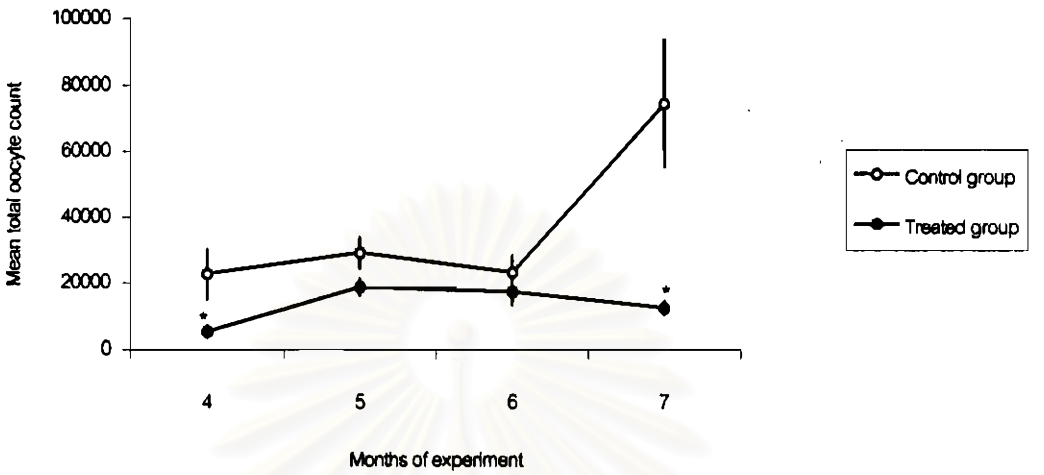



Figure 4-6 Mean (±SE) total number of oocytes in control and treated tilapia of each experimental period, *indicate significant differences from respective controls ($p \leq 0.05$).

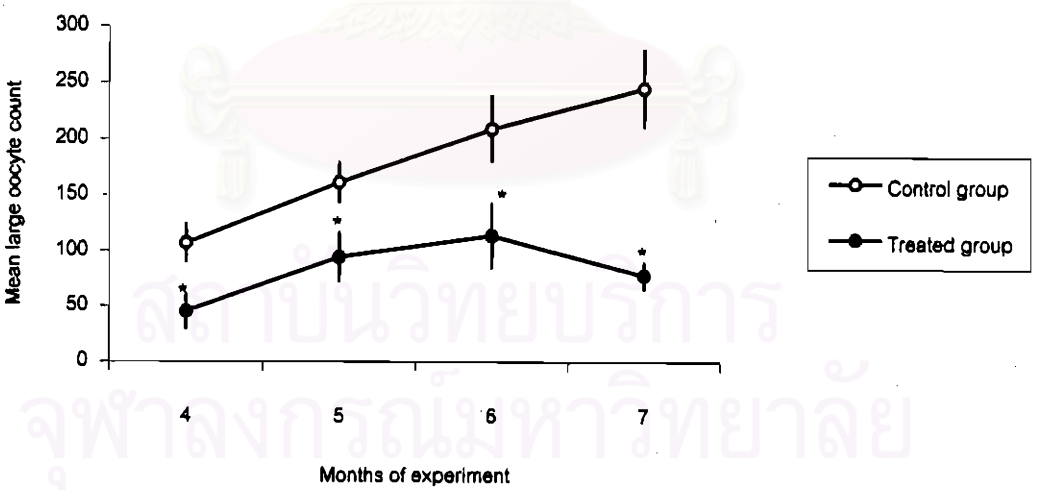



Figure 4-7 Mean (±SE) number of the large oocytes in control and treated tilapia of each experimental period, *indicate significant differences from respective controls ($p \leq 0.05$).

Table 4-2 Mean number of large, intermediate and small oocytes of Nile tilapia of control and treated group in different experimental periods. All values are shown in mean±SE.

| Size groups | Experimental groups | Experimental periods (month) | | | |
|---|---|---|---|---|---|
| | | 4th | 5th | 6th | 7th |
| Large | Control | 107±17 (n=19) | 161±18 (n=20) | 208±28 (n=21) | 244±34 (n=21) |
| | Treated | 45±15* (n=20) | 94±22* (n=19) | 114±29* (n=20) | 77±12* (n=21) |
| Intermediate | Control | 163±30 | 253±35 | 291±45 | 230±37 |
| | Treated | 94±19 | 194±27 | 136±31* | 110±19* |
| Small | Control | 22429±7583 | 28702±4652 | 22660±4905 | 73796±18906 |
| | Treated | 5169±1884* | 18486±2529 | 17128±4117 | 12308±2080* |

* Indicate significant differences from respective controls ($p \leq 0.05$).

Dey and Bhattacharya (1989) demonstrated the chronic effects of elsan, mercury and ammonia on ovary of *Channa punctatus*. A remarkable decrease in the number of mature oocytes was also found to be consistent with the result of this study on *O. niloticus*. However, ovarian toxicity of neem products on fish is still very limited.

The significant decrease in total oocyte counts and number of the ripe oocytes were well indicated female reproductive failure occuring in the developmental process of ovary and spawning of treated fish. From the results, therefore it was seen that the fecundity of female *O. niloticus* was altered after the subchronic exposure to neem seed extract.

4.2.3 Histological analysis

1. Basic histology of *O. niloticus* ovary

Ovarian tissue of the control fish at the age of 4 to 8 months were examined. Developmental stages of oocytes presented in each ovary were determined according to West (1990) as followed.

- Chromatin nucleolar stage
- Perinucleolar stage
- Yolk vesicle (cortical alveoli) formation stage
- Vitellogenic (yolk) stage
- Ripe (mature) stage

Histological characteristics of ovarian tissues and oocyte stages were studied. The observation via light microscopy revealed different histological structure of each oocyte developmental stage.

*Chromatin nucleolar stage*. The oocyte was small spherical cell containing a central nucleus. The nucleus contained one to four nucleoli together with chromatin network. Cytoplasm was thin layer and strongly basophilic. Follicular cell was difficult to see (Figure 4-8A).

*Perinucleolar stage*. The number of nucleoli increased and arranged along the inner side of nuclear membrane. Nucleus was large and surrounded by increased mass of cytoplasm which appeared less basophilic. Follicular cells was monolayer of simple squamous lining surrounded the oocyte (Figure 4-8B).

*Yolk vesicle (cortical alveoli) formation stage*: This stage is characterized by the appearance of clear vesicles (cortical alveoli) in the cytoplasm. The vesicle was begun to accumulate from the periphery of the oocyte. The nuclei were still perinucleolar. The nuclear membrane began to be convoluted. In this stage, a thin acidophilic *zona radiata* or primary envelope became visible for the first time. Follicular

layers were also seen at the first time to consist of simple cuboidal or columnar layer surrounded with pseudostratified squamous thecal layer (Figure 4-8C).

*Vitellogenic (yolk) stage*: The oocyte size increased. Small yolk granules were visible as a ring of deep eosinophilic in the cytoplasm and later incorporated the whole cytoplasmic area. The nucleus was still convoluted. The zona radiata was clearly visible as a noncellular deep eosinophilic band. Follicular layers were well-developed simple cuboidal or columnar layer surrounded by stratified squamous thecal layer (Figure 4-8D and 4-8F).

*Ripe (mature) stage*: The stage was characterized by the enlargement of both cortical alveoli and yolk granules. The oocyte size markedly increased. The peripheral migration of the nucleus was observed. The zona radiata was clearly visible. Follicular cells were cuboidal or low cuboidal surrounded by thin thecal layer (Figure 4-8E and 4-8F).

Ovarian interstitial tissues were found to consist of interstitial cells, adipose cells, yolk granules and blood capillaries (Figure 4-9). Oogonial cyst, cyst of early meiotic oocytes, was noted in ovarian tissues of the fish at the age of 5 months (Figure 4-9D). Postovulatory structure, *corpora lutea* was also observed in the ovary of the fish at the age above 6 months in both experimental groups (Figure 4-9A). The convoluted structure was found to consist of swollen granulosa cells at the inner most, surrounded with thecal cells and blood capillaries (Figure 4-9B)

2. Histological alterations of *O. niloticus* ovary

The histological changes in ovarian tissues of the treated group were observed in different level of severity depending on the exposure times as shown in table 4-3 and table 4-4.

After the exposure of neem seed extract for 4 months, the ovaries of the treated fish were observed to occupy with empty interfollicular space while the interfollicular space of the control ovaries was filled with interstitial tissues. Abnormal shape of oocytes was found as well as shrinkage of cytoplasmic borders in the perinucleolar, cortical alveolar and vitellogenic stage. Hyperbasophilic chromatin nucleolar and perinucleolar oocytes were found. Eccentric nucleus was seen in the abnormal vitellogenic oocyte. Infiltration of lymphocytes and granulocytes into interfollicular area and into ovarian capsule were evidenced severely in this exposure period. Atresia of yolked oocytes was also observed with inflammation in the area (Figure 4-10 and 4-11).

After the exposure of neem seed extract for 5 months, interfollicular space without deposition of any yolk granules or adipose cells was observed while in the control group, accumulation of yolk materials was presented in the interfollicular space. Ripe oocyte was absent in all specimen and postovulatory follicles were seen in two specimens investigated. The rest three specimens without any sign of ripe oocyte or postovulatory follicles indicated that oocyte developmental process was affected. The abnormal shape of maturing oocytes and hyperbasophilic immature oocytes were also found with shrinkage of cytoplasmic borders and damage of follicular layers. Leakage of yolked oocyte was seen with pyknotic follicular cells. Inflammation throughout the ovary was shown by the infiltration of lymphocytes and granulocytes (Figure 4-12).

After the exposure of neem seed extract for 6 months, large empty interfollicular space was observed in the treated group. Ripe oocytes were found in smaller size than that of control. Atretic yolked oocyte was evidenced as well as the abnormal shape of maturing oocytes and damage of follicular layers. Inflammation was also found in ovarian interstitial tissues and ovarian capsule (Figure 4-13).

After the exposure of neem seed extract for 7 months, empty interfollicular space was observed and in some specimen the amorphous homogeneous substance was seen in the space. Ripe oocytes were seen in this exposure period but not as large as that of control. Atresia of yolked oocyte was also observed. The abnormal shape of maturing oocytes and hyperbasophilic immature oocytes were still observed with shrinkage of cytoplasmic borders and some damage in follicular layers. The hyperbasophilic immature oocytes in some specimens were found to have an empty space in their cytoplasm resulted in allocation of nucleus to periphery of the cell. Inflammation of interstitial tissues was also detected (Figure 4-14).

Intraoocytic deposition of yolk granules and yolk materials was always seen in lower level in the treated group compared with the respective controls. Extraoocytic deposition of yolk granules and yolk materials was found markedly in lower level in the treated fish in 5 months of exposure. These results were correlated with the absence of ripe and vitellogenic oocytes from the ovaries of the treated group. Adipose interstitial tissue was seen in some ovarian specimens of the control group (all specimens in the 5th month) while it was not seen in the treated group in 4 and 5 months of exposure and in lower level in the 6 and 7 months of exposure.

Histopathological alterations in fish resulting from toxicity of neem products have been limited in literature. According to Tangtong (1997) and Janart (1997), haematological alterations and hepatotoxicity of the neem extract (Neemix®) to *O. niloticus* were reported, respectively. As indirect evidences in support of the hepatic origin of fish vitellogenin was provided by a number of studies, it is generally accepted that the liver are stimulated to synthesize and secrete vitellogenin (Ho,1987). Therefore, histopathological alterations reported in the liver of *O.niloticus* after subchronic exposure to neem extract are implied hepatic dysfunction and may affect the synthesis and secretion of vitellogenin in the treated fish. This is consistent with the present study that yolk (including vitellogenin) deposition in ovaries of the neem treated fish was markedly

lower than that of normal fish. Vitellogenin level is an indicator of female reproductive health in fish (Lye et al., 1997). Therefore, the lower accumulation of vitellogenin in the ovary of the neem treated fish indicates that female reproductive health of the fish is altered after subcronic exposure to the neem extract.

The observation of large empty interfollicular spaces and atresia of yolked oocytes of neem treated *O. niloticus* is similar to the histopathological effects in giant gaurami *Colisa fasciatus* following nickel intoxication observed by Nath and Kumer (1990). This previous study also found that ovary of the fish after 96 hours exposure to 64 ppm nickel exhibited large interfollicular spaces and atretic oocytes. The empty interfollicular space is a result from absence of interstitial tissues and correlated with degeneration of follicular cells observed in the present study. From this result, oocyte maturation may be altered because the follicular cells pay an important role to deposit yolk and secrete hormones expecially estrogen which is known to be the inducer of vitellogenesis in fish (Leake, 1975; Ho, 1987).

Oocyte in several maturing stages with abnormal shape and the hyperbasophilic immature oocyte with shrunken cytoplasmic borders found in the ovaries of neem treated tilapia are the evidences of unavailability and imminent degenertion. These results indicate that oocyte developmental process was impaired. The degeneration of immature oocytes was similarly observed in *Channa punctatus* exposed to ammonium sulfate for 6 months (Ram and Sathyanesan, 1986). However, observation of abnormal shape and shrunken oocytes in some cases in this study may be resulted from compression and distortion by inappropriate slide prepartion.

Infiltration of lymphocytes and granulocytes is an evidence of chronic inflammation occurred by the long-term injury in the ovaries of the treated fish. From the sites observed to be inflammed, it can be said that there were previous injuries. This

result supports that the ovarian interstitial tissues and the ovarian capsule of the neem treated fish were damage.

By histological observation, it also revealed that the number of mature oocytes presented in each sections was lower in treated fish. This is consistent to the result of large oocyte counting (Figure 4-7) which revealed the significant low number of the oocyte in the ovaries of treated fish. The number of ripe oocytes presented in the ovary represents the number of eggs spawned. From this reason, spawning of the treated fish could be disturbed by the exposure to the neem extract.

Table 4-3 Histological events in Nile tilapia ovarian tissues of each experimental group indicated as different levels of severity*.

| Experimental month/group/no. | Histological lesions | | | | | |
|---|---|---|---|---|---|---|
| | Yolk deposit | | Adipose tissues | Empty Interfollicular space | Atretic follicle | Inflammation |
| | Intraoocytic | Extraoocytic | | | | |
| 4th/control/1 | ++ | - | - | - | - | - |
| 4th/control/2 | ++ | - | + | - | - | - |
| 4th/control/3 | ++ | - | - | - | - | - |
| 4th/control/4 | ++ | ++ | ++ | - | - | - |
| 4th/control/5 | ++ | - | - | - | - | - |
| 4th/treated/1 | - | - | - | ++ | - | ++ |
| 4th/treated/2 | - | - | - | ++ | - | ++ |
| 4th/treated/3 | + | - | - | ++ | - | ++ |
| 4th/treated/4 | + | - | - | ++ | + | ++ |
| 4th/treated/5 | + | - | - | ++ | - | ++ |
| 5th/control/1 | ++ | ++ | ++ | - | - | - |
| 5th/control/2 | ++ | - | ++ | - | - | - |
| 5th/control/3 | ++ | ++ | ++ | + | - | ++ |
| 5th/control/4 | ++ | ++ | ++ | - | - | - |
| 5th/control/5 | ++ | ++ | ++ | ++ | - | - |
| 5th/treated/1 | + | + | - | + | ++ | ++ |
| 5th/treated/2 | + | - | - | ++ | ++ | ++ |
| 5th/treated/3 | + | - | - | ++ | - | ++ |
| 5th/treated/4 | + | - | - | ++ | - | - |
| 5th/treated/5 | + | - | - | ++ | - | ++ |

* - not observed, + mild, ++ severe

Table 4-3 Histological events in Nile tilapia ovarian tissues of each experimental group indicated as different levels of severity* (cont.).

| Experimental month/group/no. | Histological lesions | | | | | |
|---|---|---|---|---|---|---|
| | Yolk deposit | | Adipose tissues | Empty Interfollicular space | Atretic follicle | Inflammation |
| | Intraoocytic | Extraoocytic | | | | |
| 6th/control/1 | ++ | + | ++ | - | - | - |
| 6th/control/2 | ++ | ++ | ++ | - | + | - |
| 6th/control/3 | ++ | - | - | - | - | - |
| 6th/control/4 | ++ | - | ++ | - | + | - |
| 6th/control/5 | ++ | ++ | - | - | - | - |
| 6th/treated/1 | ++ | + | - | ++ | - | ++ |
| 6th/treated/2 | ++ | ++ | + | ++ | + | ++ |
| 6th/treated/3 | + | - | - | ++ | - | - |
| 6th/treated/4 | - | - | - | ++ | - | ++ |
| 6th/treated/5 | - | - | - | ++ | - | ++ |
| 7th/control/1 | ++ | - | - | - | + | - |
| 7th/control/2 | ++ | - | ++ | - | + | - |
| 7th/control/3 | ++ | + | ++ | - | - | - |
| 7th/control/4 | ++ | ++ | + | - | - | - |
| 7th/control/5 | ++ | - | + | - | - | - |
| 7th/treated/1 | ++ | - | + | + | - | - |
| 7th/treated/2 | ++ | + | + | + | ++ | - |
| 7th/treated/3 | ++ | + | - | + | ++ | - |
| 7th/treated/4 | ++ | - | - | - | - | ++ |
| 7th/treated/5 | + | - | + | + | ++ | - |

* - not observed, + mild, ++ severe

Table 4-4 Histological alterations of oocyte in the ovary of each experimental group indicated as different levels of severity*.

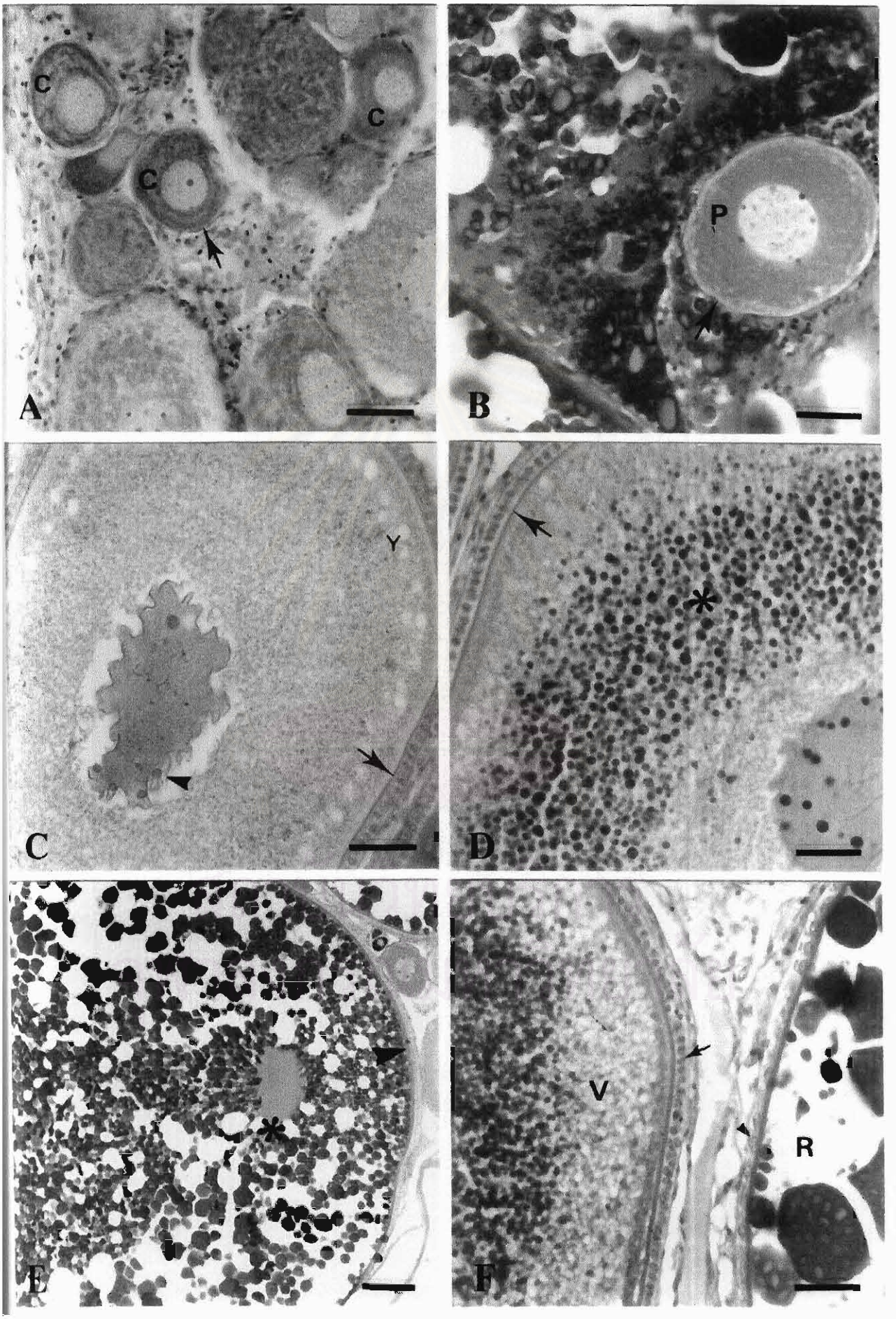
| Experimental month/group/no. | Histological lesions | | | | |
|---|---|---|---|---|---|
| | Mature oocytes | Hyperbasophilic Oocyte | Oocyte with shrunken cytoplasmic borders | Oocyte with Abnormal shape | Degeneration & blebling of follicular layer |
| 4th/control/1 | + | - | + | + | - |
| 4th/control/2 | ++ | - | + | - | - |
| 4th/control/3 | ++ | - | + | - | - |
| 4th/control/4 | ++ | - | - | - | - |
| 4th/control/5 | ++ | - | - | - | - |
| 4th/treated/1 | - | - | ++ | ++ | + |
| 4th/treated/2 | - | - | + | + | + |
| 4th/treated/3 | - | - | ++ | ++ | ++ |
| 4th/treated/4 | - | ++ | - | ++ | ++ |
| 4th/treated/5 | - | ++ | ++ | ++ | ++ |
| 5th/control/1 | ++ | - | + | - | - |
| 5th/control/2 | ++ | - | - | + | - |
| 5th/control/3 | ++ | - | + | + | + |
| 5th/control/4 | ++ | + | + | - | - |
| 5th/control/5 | ++ | - | + | + | - |
| 5th/treated/1 | - | ++ | - | ++ | ++ |
| 5th/treated/2 | - | ++ | ++ | ++ | ++ |
| 5th/treated/3 | - | ++ | ++ | ++ | ++ |
| 5th/treated/4 | - | ++ | ++ | ++ | ++ |
| 5th/treated/5 | - | ++ | ++ | ++ | + |

* - not observed, + mild, ++ severe

Table 4-4 Histological alterations of oocyte in the ovary of each experimental group indicated as different levels of severity* (cont.).

| Experimental month/group/no. | Histological lesions | | | | |
|---|---|---|---|---|---|
| | Mature oocytes | Hyperbasophilic oocyte | Oocyte with shrunken cytoplasmic borders | Oocyte with Abnormal shape | Degeneration & blebling of follicular layer |
| 6th/control/1 | ++ | - | + | - | - |
| 6th/control/2 | ++ | - | + | + | - |
| 6th/control/3 | ++ | - | + | + | - |
| 6th/control/4 | ++ | - | + | + | - |
| 6th/control/5 | ++ | - | + | - | + |
| 6th/treated/1 | ++ | ++ | ++ | ++ | + |
| 6th/treated/2 | ++ | ++ | ++ | ++ | + |
| 6th/treated/3 | ++ | ++ | ++ | ++ | + |
| 6th/treated/4 | ++ | ++ | ++ | ++ | ++ |
| 6th/treated/5 | ++ | ++ | ++ | ++ | ++ |
| 7th/control/1 | ++ | + | + | + | - |
| 7th/control/2 | ++ | + | + | + | - |
| 7th/control/3 | ++ | - | + | + | - |
| 7th/control/4 | ++ | - | + | + | - |
| 7th/control/5 | ++ | - | + | + | - |
| 7th/treated/1 | ++ | + | + | + | - |
| 7th/treated/2 | ++ | ++ | ++ | ++ | ++ |
| 7th/treated/3 | ++ | ++ | ++ | ++ | ++ |
| 7th/treated/4 | ++ | ++ | ++ | ++ | + |
| 7th/treated/5 | - | ++ | ++ | ++ | - |

* - not observed, + mild, ++ severe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V
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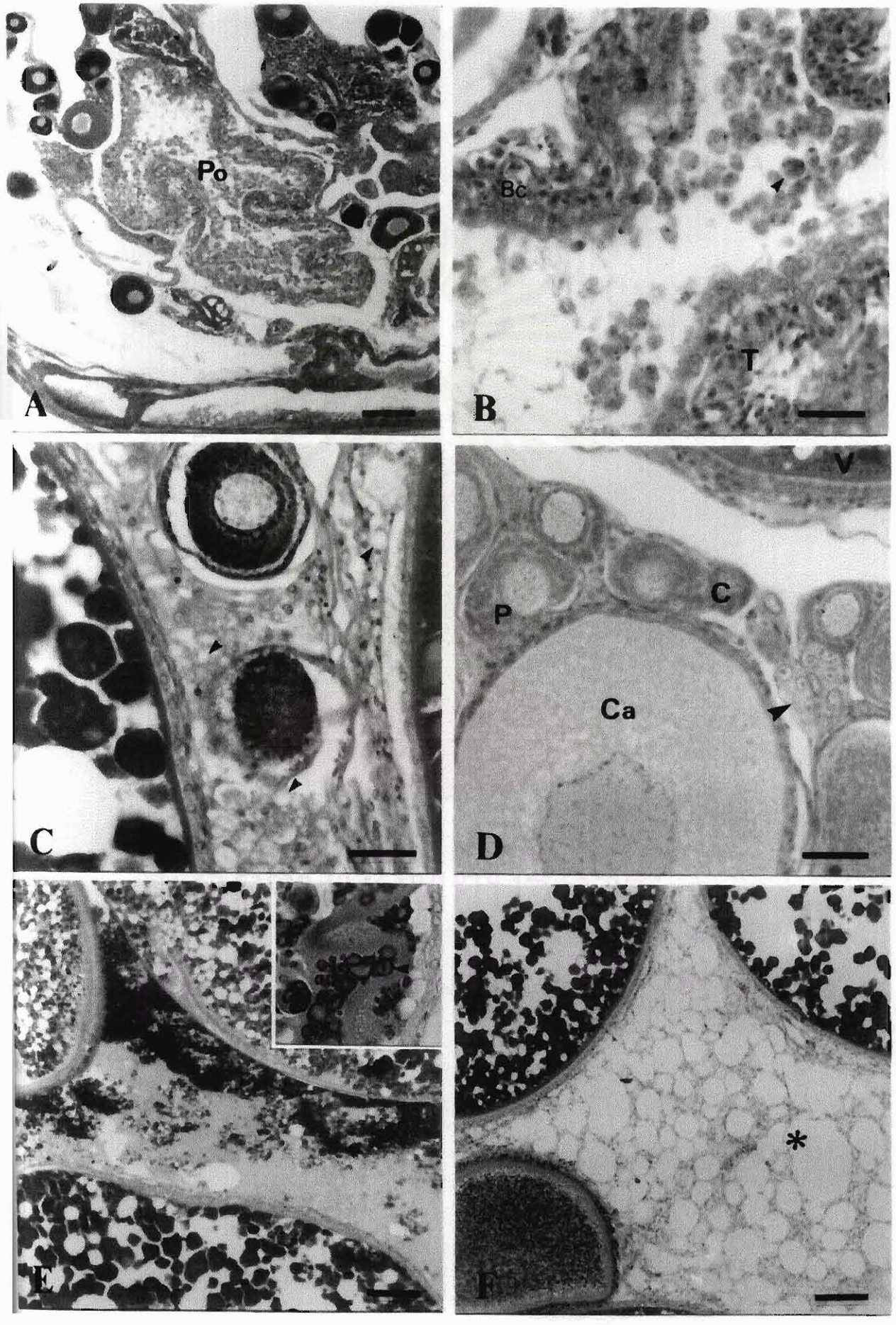
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A
Bc
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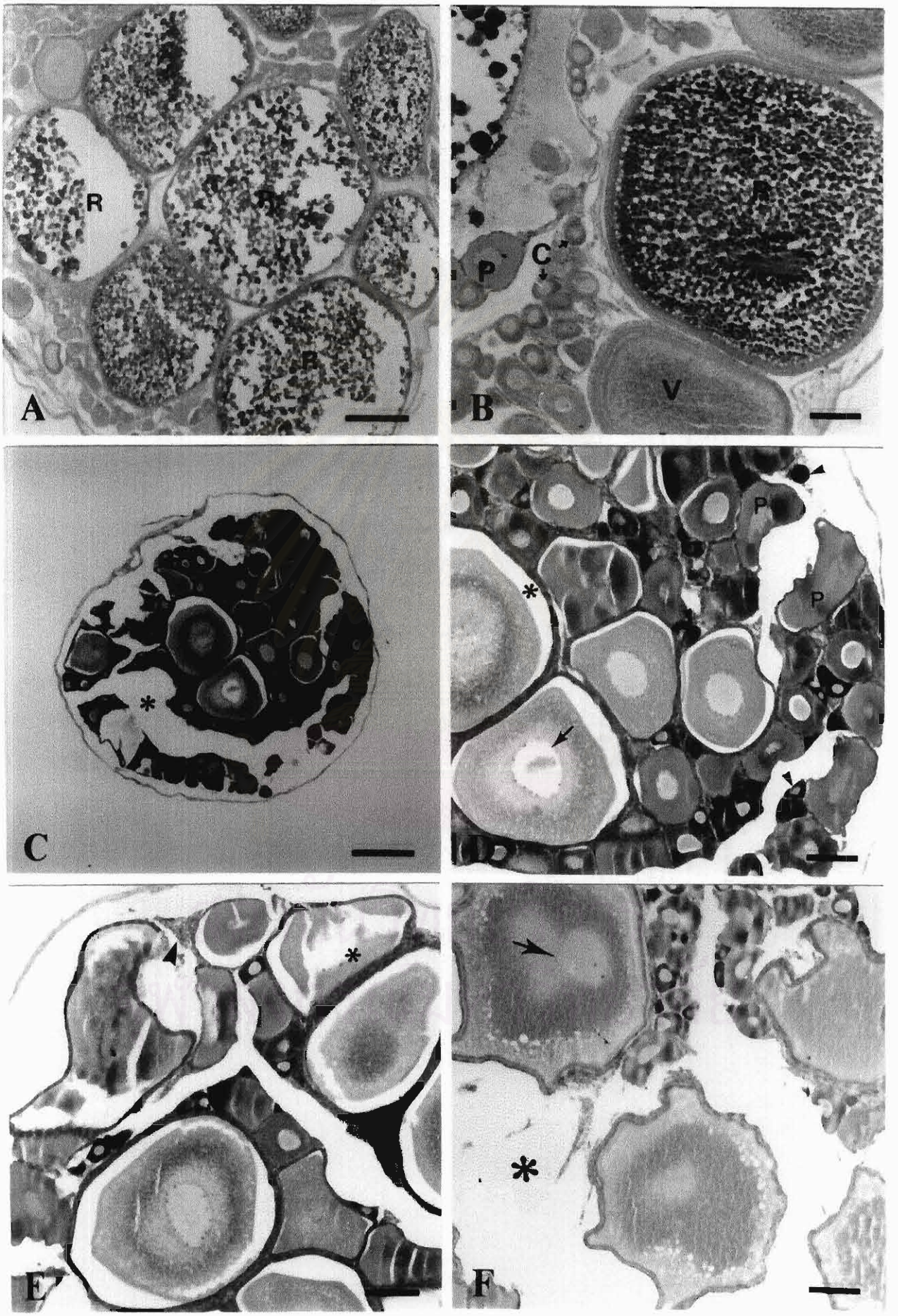
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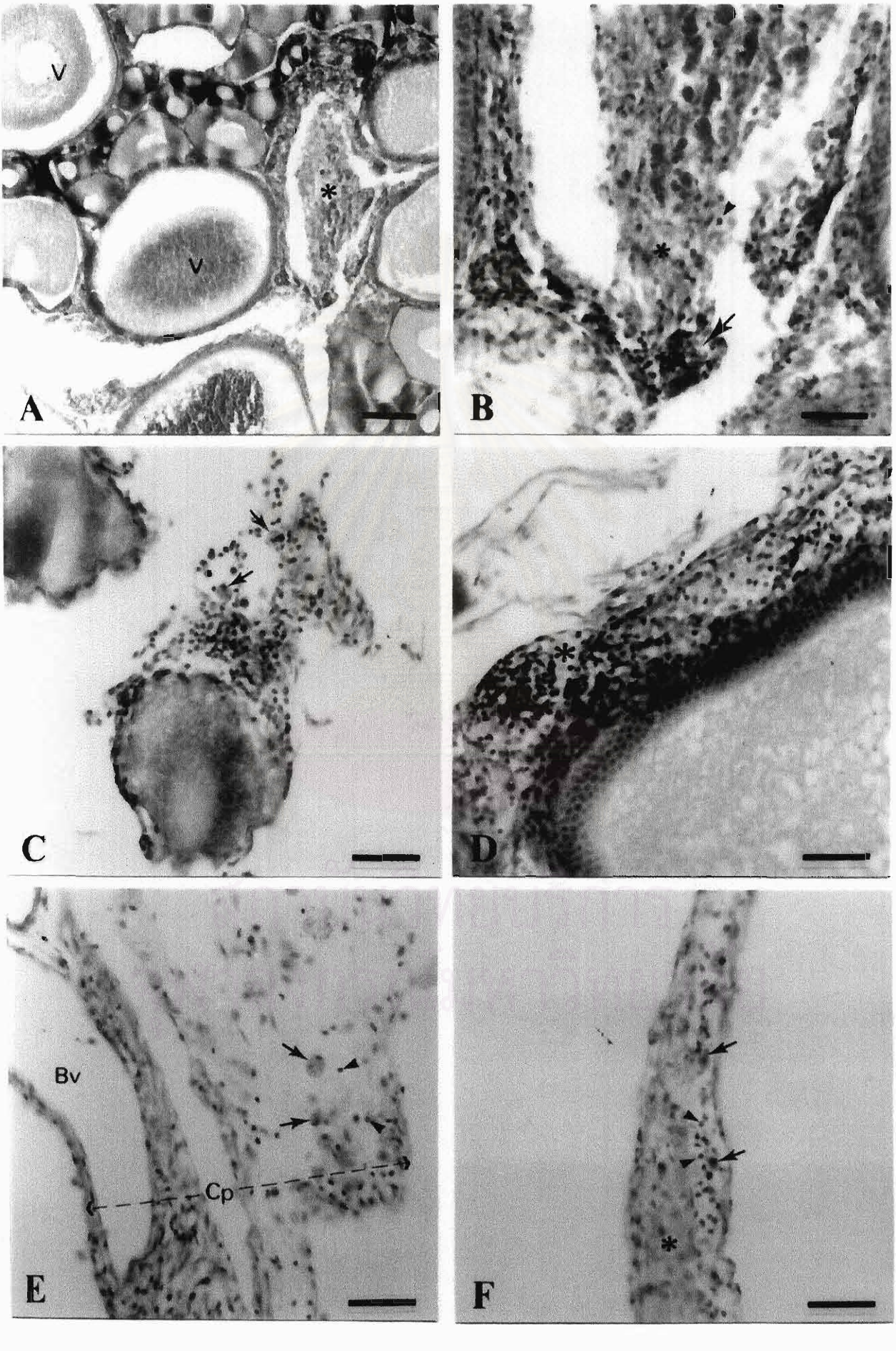
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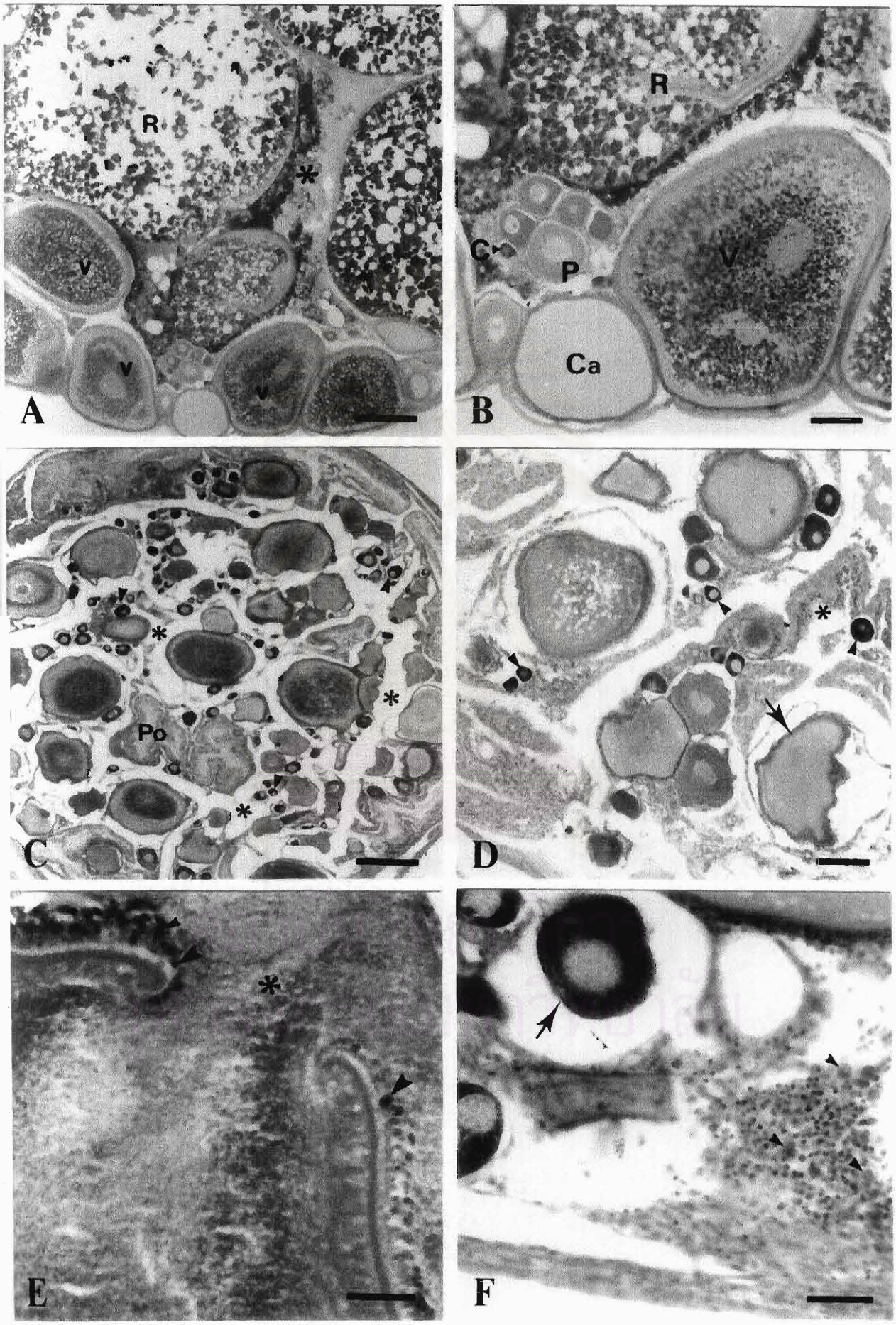
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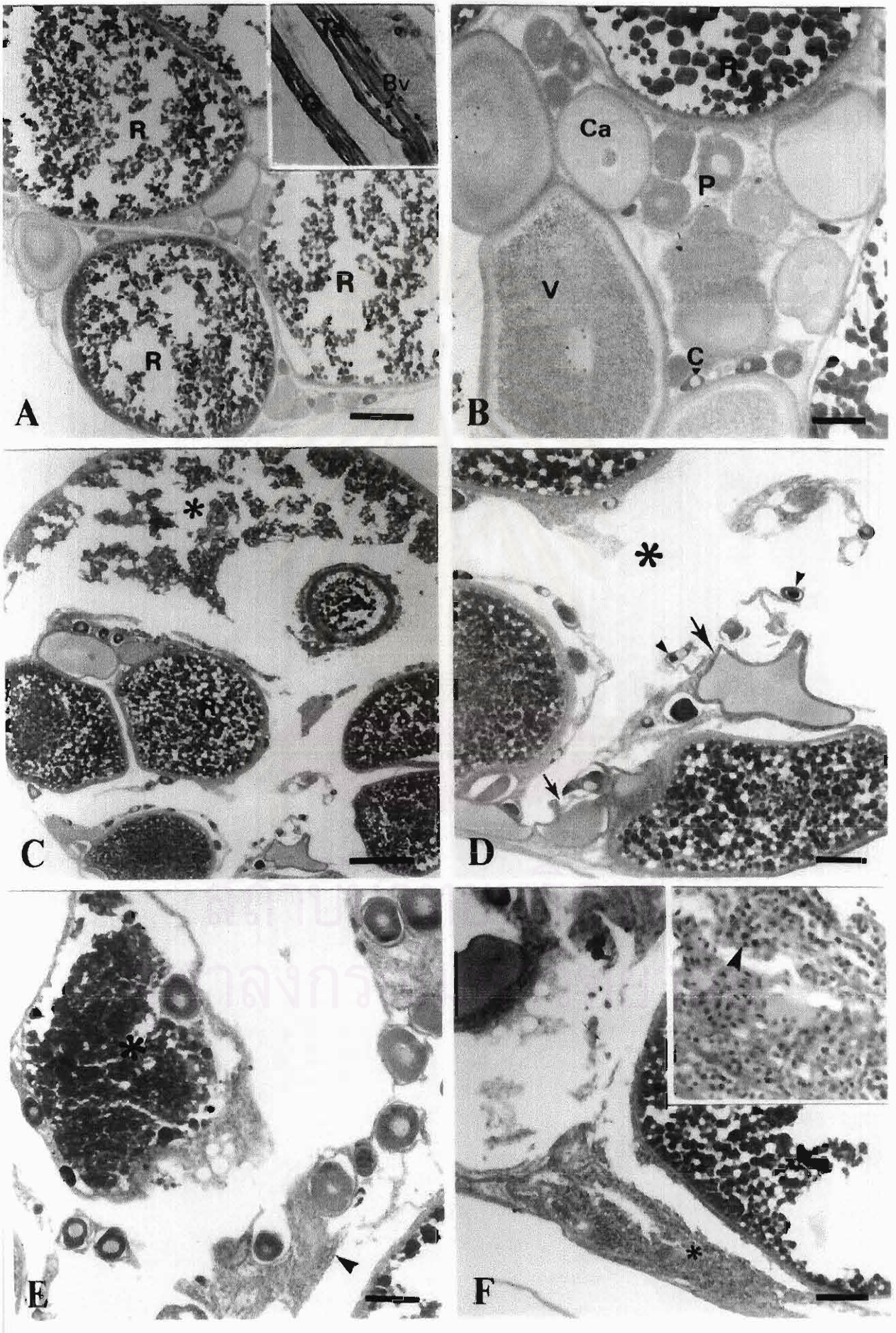
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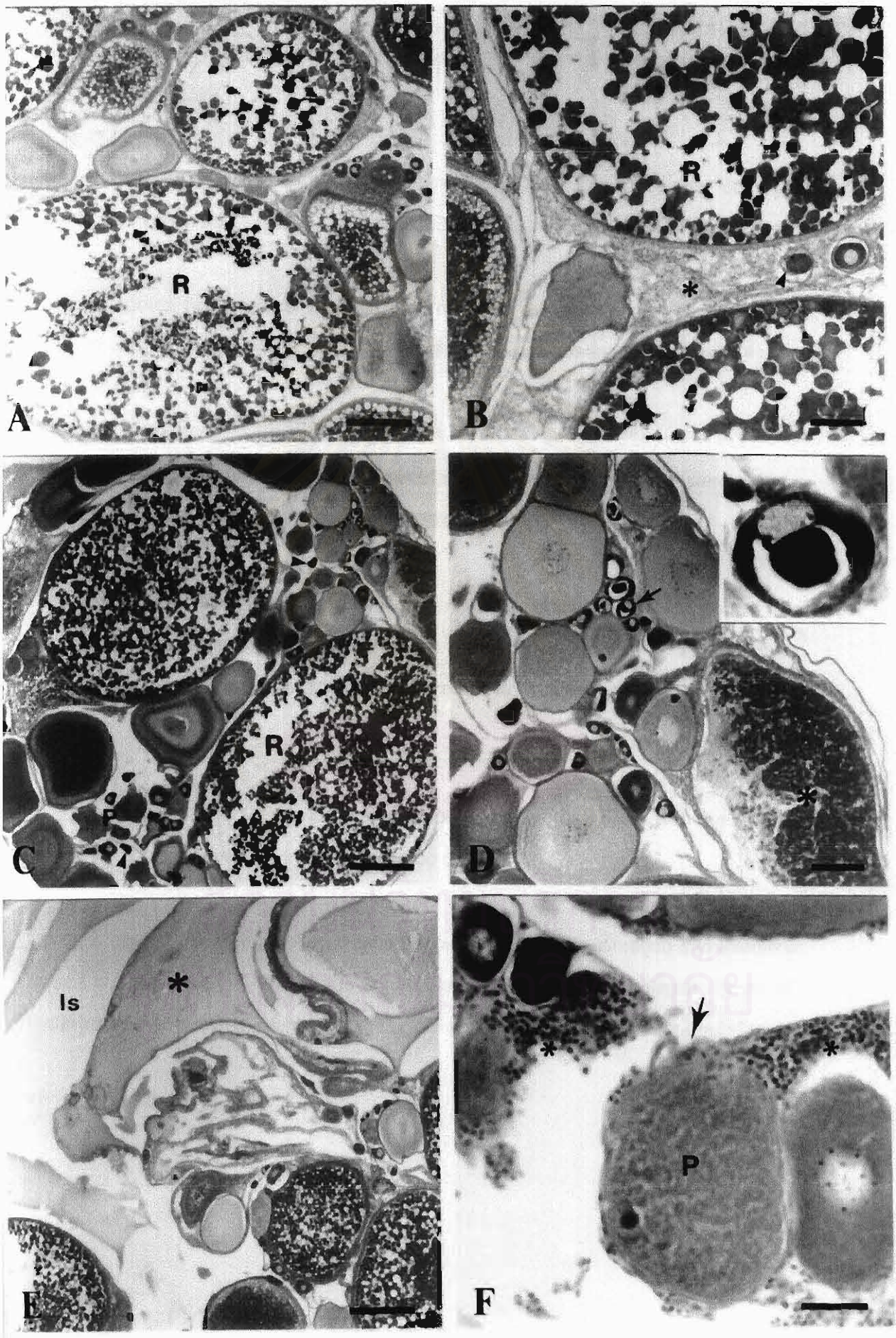
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Figure 4-14 Photomicrograph of *O. niloticus* ovaries at 7 months of experimental period (H&E stain).

A. Ovary of control fish shows the normal structure of oocytes in several stages of development including large ripe oocyte (R). Bar scale = 300 μm.

B. Ovary of control fish in higher magnification contains immature oocyte (➢) and large ripe oocyte (R). Interfollicular space is filled with ovarian interstitial tissues (*). Bar scale = 100 μm.

C. Ovary of treated fish shows small ripe oocyte (R), hyperbasophilic immature oocytes (➢) and shrinken perinucleolar oocyte (P). Bar scale = 300 μm.

D. Ovary of treated fish in higher magnification shows atretic yolked oocyte (*) and numerous hyperbasophilic immature oocytes with space formation in the cytoplasm (➢). Inset shows the hyperbasophilic immature oocyte with space formation. Bar scale = 100 μm.

E. Ovary of treated fish shows large interfollicular space (Is) filled with amorphous homogeneous substance (*). Bar scale = 300 μm.

F. Ovary of treated fish in high magnification shows lymphocyte infiltration into interstitial tissues of the ovary (*) and damage of follicular layer (➢) of perinucleolar oocyte (P). Hyperbasophilic oocytes are seen at the upper left corner. Bar scale = 30 μm.