Bull. Nat. Inst. Oceanogr. & Fish. ARE, 15,(2), 1989 : 237 - 251

HISTOLOGICAL CHANGES IN OREOCHROMIS NILOTICUS (E. TREWAVAS, 1982), DEVELOPING OVARIES DUE TO ORAL ADMINISTRATION OF ETHINYLESTRADIOL AND NORETHINDRONE.

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ABSTRACT

Histological changes in the ovary of **Oreochromis niloticus**, were studied due to oral administration of a combined dose of ethinylestradiol and norethindrone; in the pill norminest. Increase in the hormonal dose increased the formation of acidic proteins due to estrogen effect; and attresia due to feedback mechanism. Nuclei of the germinative cells played a role in this process. Autolysis is complete among fish of 7 cm, and partial among fish of 7 cm in total length. The right side ovary lobe was more effected than the left one.

INTRODUCTION

Human contraceptive pills have been used by poultry farm owners to improve growth in weight. It is a consequence that this procedure might be applied in the growing industry of fish farms. With lack of education or even information regarding fish farming, the use of polls may even spread to larger fish and not only the fish fry.

Steroids have been used by some workers to control Tilapia reproduction throughout sex reversal (Koplin et al., 1977; Tayamen and Shelton, 1978; Guerrero, 1979 and Hopkins et al., 1979). Eckestin and Spira (1965) were the first in using estrogens at concentrations of 50 to 100 ug per litre of aquarium for 3 to 4 weeks, to destroy the gonads of S. aureus (Tilapia aurea) fry. It is for the first time, however, in this study that the effects of a combined dose of the steroids, ethinylestradiol and norethindrone, in the commonly used pill "Norminest" upon maturing fish of female Tilapia nilotica (Oreochromis niloticus) are going to be assessed.

MATERIAL AND METHODS

Two collections of fish specimens, from Bahr Shebeen canal, were used for treatment. The first ranged between 3 to 5 cm and the second was between 7 to 10 cm in total length. The experiment durated for about one month; August for the first and June for the second. Fish were kept in five glass aquaria with the dimentions: 58.5 x 30 x 40 cm

for each aquarium. High mortality was found on using tap water but survival stabilized after using a mixture of 2/3 Bahr Shebeen water plus 1/3 of tap water, then one third of the first to 2/3 of the second, and finally 100 % tap water for two days for each change. Each aquarium had an aeration pump operating continuously, except 20 minutes two times every day on feeding the fish.

The pill norminest contains 0.5 mg norethindrone (Progestin equivalent) and 0.35 ug ethinylestradiol (Estrogen equivalent). The dose per litre of aquarium water was prepared, for aquaria I to IV, by using concentrations 1, 2, 3 and 4 times the original dose respectively. Aquarium V was used as a control.

After Guerrero (1982), Hunter et al. (1982) and Goudie et al. (1983), the required number of pills was ground and dissolved in 250 ml of 95 % ethyl alcohol and mixed with 250 gm commercial food then dried for one hour at 60°C.

Gonads were preserved in Bouins fixative with labels recording the fish total length, standard length to the nearest cm and weight to the nearest gram. Embedding was carried out in parablast parafin wax and sections were 6 to 8 microns. Staining was carried out by Ehrlich hematoxylin or hematoxylin and eosin (Humason, 1979).

RESULTS

The process of oogenesis of O. niloticus was described in detail earlier by Latif and ElSaady (1973). However, Figures 1 to 3 show various stages of this process for comparison. The effect of treatment upon ovary development is going to be examined as follows:

Aquarium I (35 ug ethinylestradiol + 0.5 mg norethindrone):

Examined specimens till two weeks from the experiment did not show marked deviation from the normal state. However, specimens dissected after 26 days were found to have irregularity in the gonad shape, and the oldest generation of eggs is undergoing attresia (Fig. 4). These eggs may range in diameter between 180 to 220 u for the long axis and 100 to 130 u for the short axis. The nuclei in these eggs are about 40 u in diameter. Nucleoli are peripheral lying close to the nuclear membrane. The cytoplasm of these eggs is vacuolated, although these eggs are in the state of yolk formation. The yolk in these eggs is stained violet in color as compared with the reddish or pinkish color of the egg components. The follicle around each egg of this type is loose and flappy. The eggs of the second generation are about 100 to 140 u for the largest diameter. Their nuclei are about 20 to 40 u in diameter, but nucleoli cannot be seen. They are dark in color when compared with the older generation of eggs. Vacuolation is also present in the cytoplasm of eggs, and the eggs are enveloped by a loose follicle. Some eggs ranging between 70 to 100 u in diameter,

are characterized by having no nuclei but an empty space instead in the center radiating in various directions. The younger generation ranges between 10 to 40 u in diameter. These are the darkest in color with small round acidophilic nuclei.

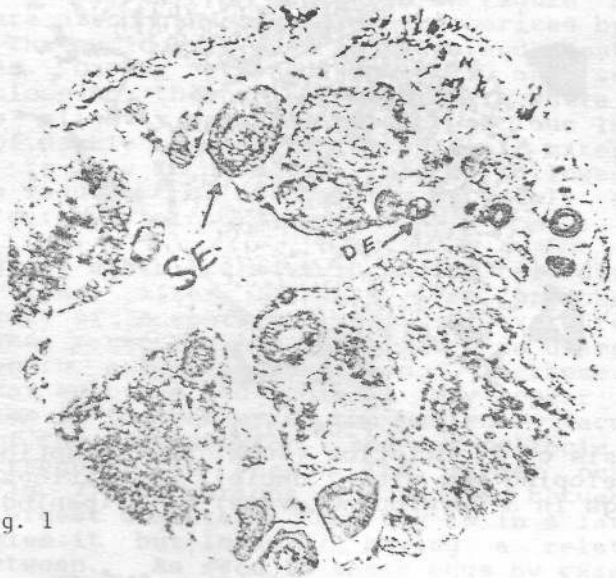


Fig. 1

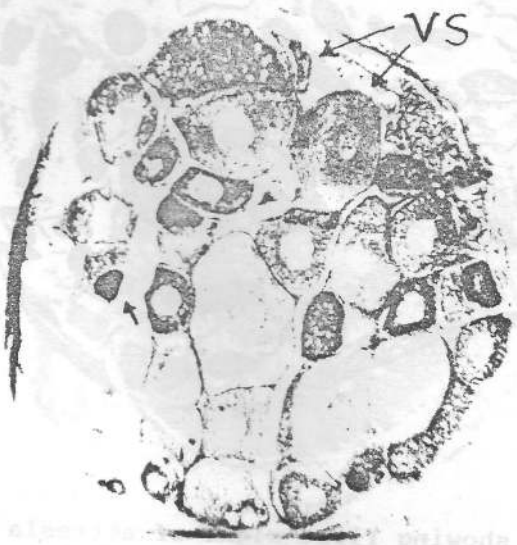
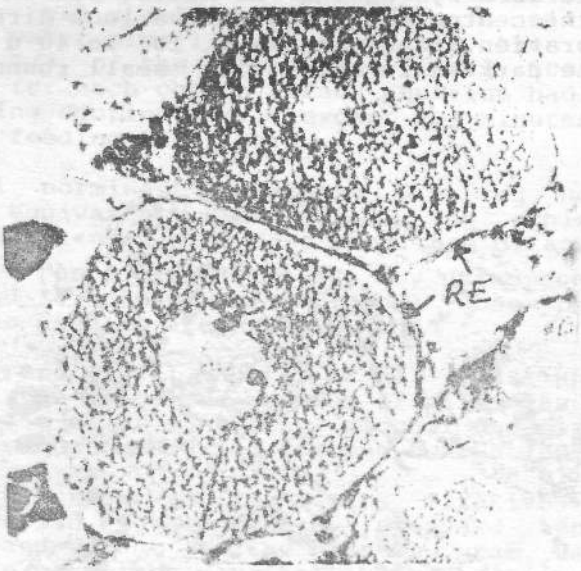


Fig. 2

Fig. 3



FIGS. 1-3
Oogenesis of T. nilotica (60 X; SE, synaptic egg; DE, developing egg with 2 unclei; VS, various stages of eggs in a developing ovary; RE, ripening egg).

FIG. 4
Ovary section showing first signs of attresia and nucleus abnormality (60 X; AE, abnormal egg; Fish L. = 9.8 cm).

Aquarium II (70 ug ethinylestradiol + 1.0 mg norethindrone):

After one week from treatment in this aquarium, the examined fish specimens have two effected ovaries with the right one in a relatively reduced state. In the smaller ovary, few eggs of the diameter 180 to 220 u are present but undergoing autolysis. As indicated in Figure 5, their egg membranes are discontinuous and characterized by basophilic granules. The nucleus is oval in shape and about 60 x 50 u in diameter. Darkly stained nucleoli are seen at the periphery close to the nuclear membrane, while the nuclear material is slightly basophilic. The nucleus is surrounded by a ring of darkly basophilic cytoplasmic material ranging between 10 to 20 u in diameter. This is followed by an area filled with vacuoles or acidophilic material, and then the peripheral darkly stained cytoplasm.

Fish ovaries exhibited more morphological differences in shape and volume, after 11 days from treatment. In the smaller ovary of a specimen (Fig. 6) very few eggs are present. They range between 50 to 220 u in diameter and are clearly seen in autolytic condition. In those eggs, there is no nuclear membrane and nucleoli may appear as numerous dark granules in the center of the nucleus. Vacules seem to fill most of the egg interior, and egg follocle if seen is loose and irregular in shape. The larger ovary of this specimen (Fig. 7) has various eggs ranging between 50 to 250 u for the largest diameter. Each egg is in a large follicle that encircles it but loosely; having a relatively large space in between. As seen in these eggs by examining their nuclei as well as their staining affinity, they do not conform into one stage of maturity.

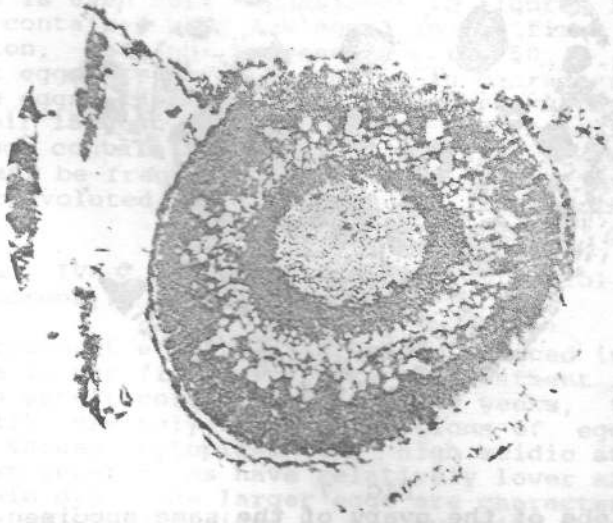


FIG. 5
An ovum undergoing autolysis in detail (200 X; L = 8.2 cm).

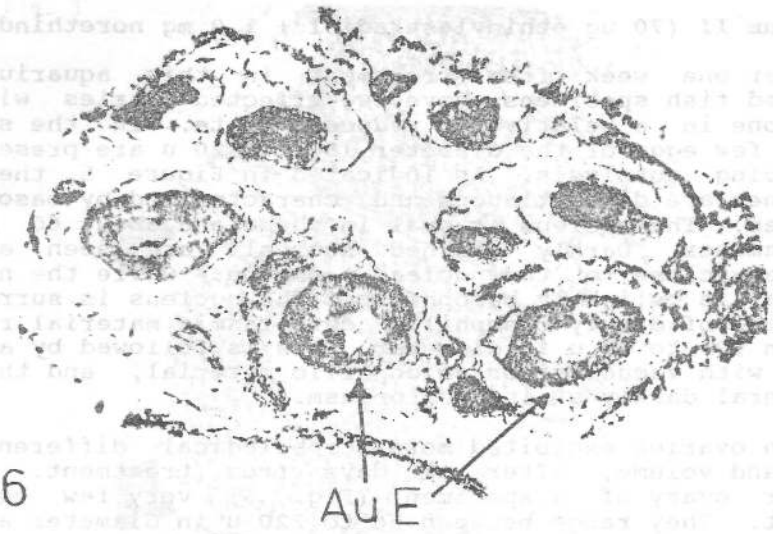


FIG. 6
Section of a small lobe of an ovary showing progressive autolysis in eggs (60 X; AuE, autolytic egg; L = 9.0 cm).

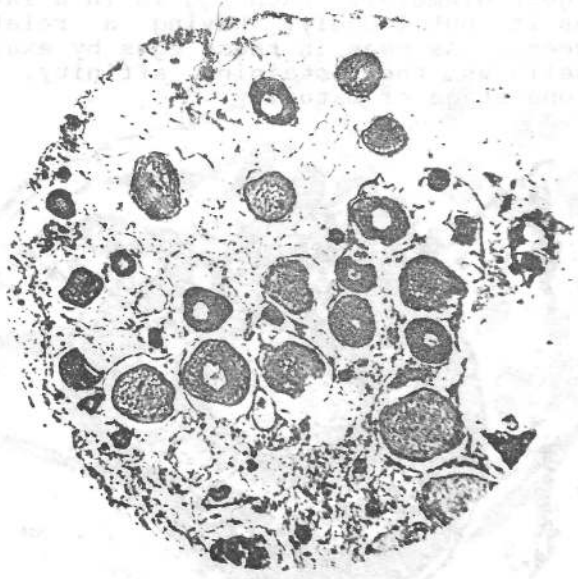


FIG. 7
Larger lobe of the ovary of the same specimen in Fig. 6 (60 X).

Aquarium III (105 ug ethinylestradiol + 1.50 mg norethindrone):

One week from the treatment, the fish has two gonads smaller in zise, but the right one is greatly smaller than the other. In a smaller ovary section (Fig. 8), few eggs are present. These range between 40 to 200 u for the largest diameter. Their follicles are loose, and they contain vacuoles while nuclear membrane may or may not be visible. The main bulk of the gonad seems to be either empty or acidophilic with fine basophilic particles, granules or thread-like structures.

After 3 weeks under treatment in this aquarium, the larger lobe of an ovary (Fig. 9) contained various types of eggs. The largest eggs never exceeded 300 u for the largest diameter, in spite of their advanced stage they are autolytic in condition. In addition to the loose follicle, the egg membrane itself is irregular in shape and the nucleus without the nuclear membrane and enveloped by a ring of dark basophilic material. Smaller eggs do not exceed 50 u in diameter, they are characterized by large nucleus about 20 to 30 u in diameter and dense basophilic cytoplasm. These eggs are not growing or ceased to grow since the follicles around them are loose. Although the ovary is bulky yet it is filled with irregularly distributed basophilic material.

In Figure 10, the smaller lobe of the ovary shows only one or two eggs in a very attretic condition. No definite structures can be identified. The whole bulk of this reduced ovary is filled with unidentified material varying in staining affinity towards basic or acidic dyes. However, a certain longitudinal space is nearly filled with an acidophilic material, as it is stained dark red by eosine. The case is even more emphasized in Figures 11 and 12. The ovaries contained very few eggs in the first or the second generation, ranging between 20 to 50 u diameter. The smallest eggs are more basophilic in character. In addition to these eggs, there are oval structures with more than 1000 u in their largest diameter. They are loose in their border and do not contain identifiable material. Vacuoles or empty spaces may be frequent inside these forms. The ovary wall is thin, convoluted and loose around its content in certain regions.

Aquarium IV (140 ug ethinylestradiol + 2.0 mg norethindrone):

No prominent effects other than noticed in the preceded aquariam in the first two weeks of treatment in the present aquarium were recorded. After three weeks, the larger lobe (Fig. 13) has only three generations of eggs. The smaller oocytes showed cytoplasm with high acidic stain while the older two generations have relatively lower affinity towards the acidic dye. The larger eggs are characterized by nuclei that are slightly stained by the dye with faintly basophilic nucleoli. The cytoplasm is vacuolated, and the whole oocyte is contained in a loose follicle.

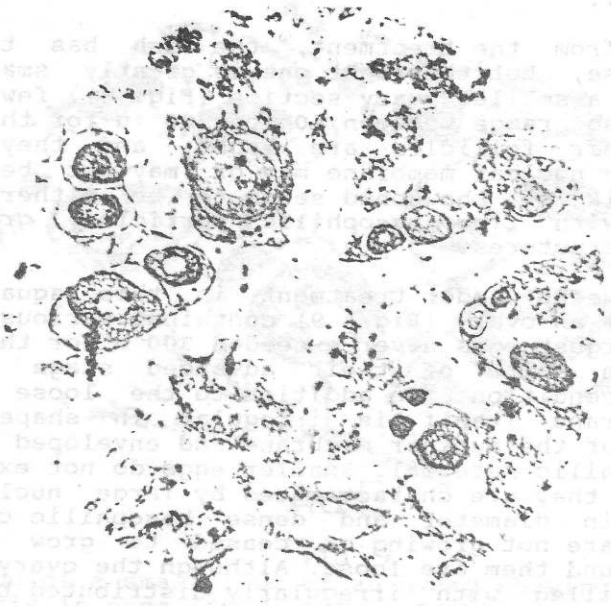


FIG. 8
Section of a smaller lobe of an ovary showing destruction of most of the ovary (90 X; L = 7.8 cm).

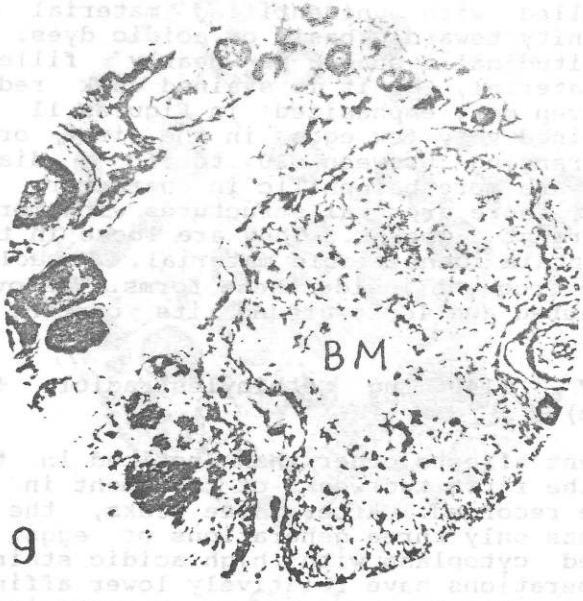


FIG. 9
Section of a large lobe of an ovary showing progressive autolysis (90 X; BM, basophilic material; L = 5.2 cm).

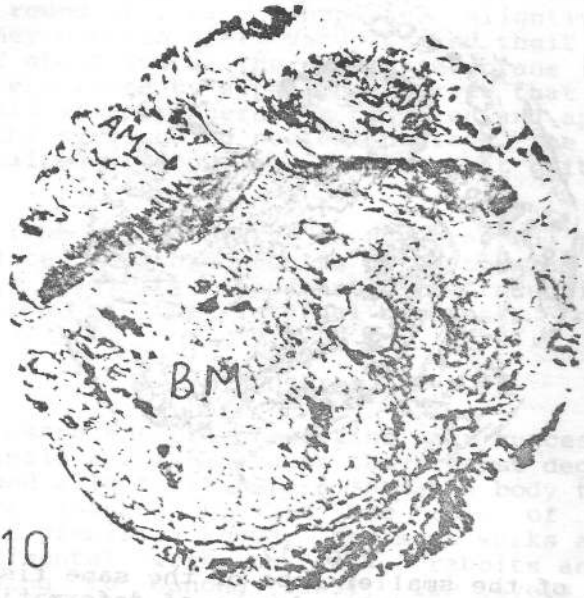


FIG. 10
Section of the smaller lobe of the same fish in Fig. 9 showing complete autolysis (90 X; AM, acidophilic material; BM, basophilic material).

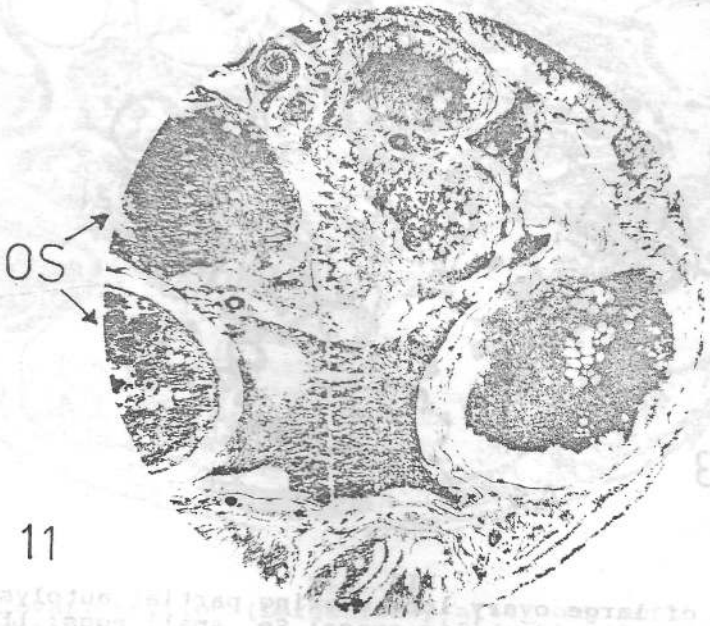


FIG. 11
Section of a large lobe of an ovary showing abnormal oval structures (OS) (60 X; L = 8.3 cm).

FIG. 12
Section of the smaller lobe of the same fish in Fig. 11 showing higher degree of deformation (60 X; OW, ovary wall).

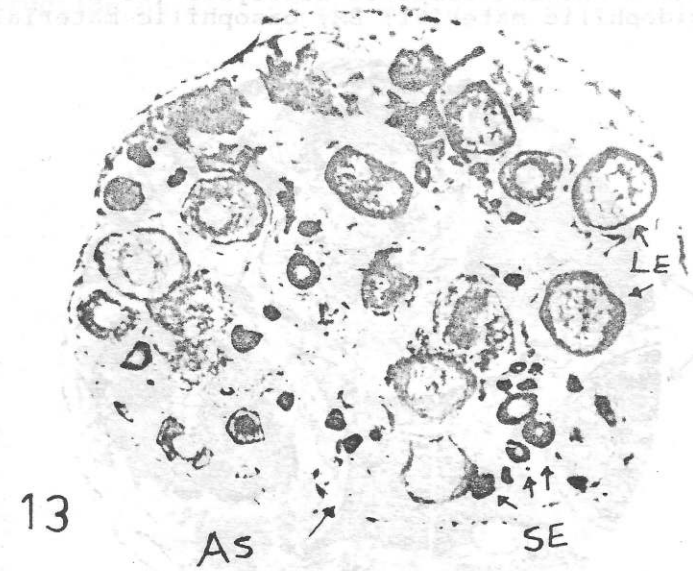


FIG. 13
Section of large ovary lobe showing partial autolysis (60 X; As, acidophilic space; Se, small eggs; LE, large eggs; L = 7.7 cm).

In Figure 14, eggs ranging between 300 and 400 u in diameter are round or oval in shape and slightly basophilic in nature. They contain small vacuoles and their nuclei have a diameter of about 100 u. The nucleus membrane is irregular in shape and encircled by a peripheral zone that acquired no stain. Nucleoli are peripheral in position and appear little darker than the surrounding nuclear content. The rest of the section contained acidophilic material but with irregular shape.

The right lobe of the ovary in the treated fish in this aquarium produced very bad results on sectioning because of its material fragility. Apparently the reduction in shape and dissolution of the internal contents caused this difficulty.

DISCUSSION

Synthetic estrogens or progestins are successfully used orally in animals and man because they resist degradation in the liver, and able to transform into the body to a steroid ring-structure (Ganong, 1983). The role of steroids in animals is not precisely known, and most works are compiled through experimental works on rats, rabbits and chickens. Thus, according to Ganong (1983), low levels of estrogen

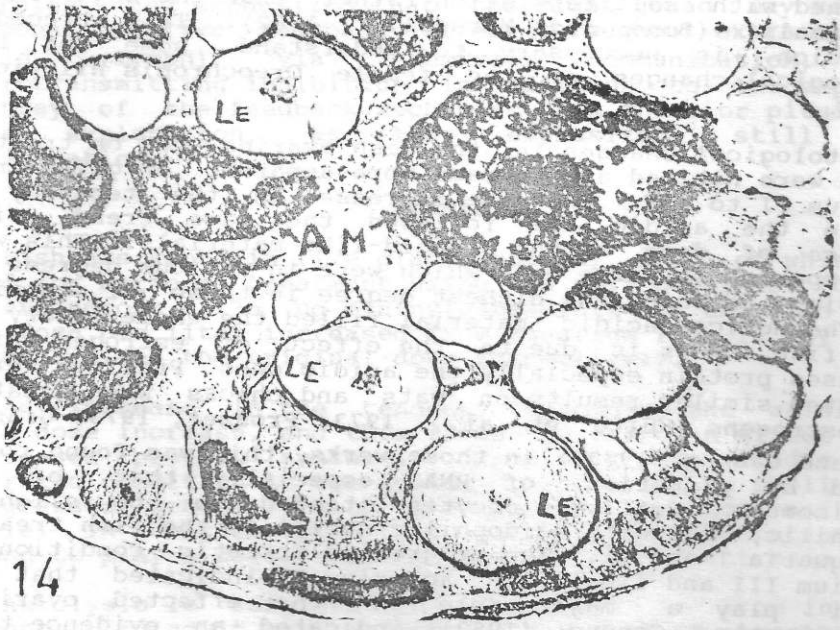


FIG. 14
An ovary section (Large lobe) after one month of treatment in aquarium IV (100 X; AM, acidophilic material; LE, large deformed egg; L = 8.6 cm).

have a positive feedback control leading to gradual increase of follicle stimulating hormone "FSH" in the early phase of the ovarian cycle. This, in turn, will cause rise in the estrogen level to trigger release of leutinizing hormone "LH". However, the high level of estrogen exerts a negative feedback control over the hypothalamus causing inhibition of FSH from the pituitary gland. This process of positive feedback control of estrogen is inhibited by high level of progestrone. This property is used in the contraceptive pills to minimize the positive feedback effect of estrogens, although progestrone is partly metabolised into estrogen. Estrogens are found to cause increase in angiotensin (I then II) which causes water retention as well as salts of sodium, calcium and phosphorus, in addition to increase in protein synthesis (Maccan et al., 1971; Prosser, 1973; Grant, 1978 and Ganong, 1983).

According to Lagler et al. (1977), Goetz and Bergman (1978) in fish there is a piscine gonadotropine PPG rather than FSH and LH of the pituitary. Another difference is also implied by Peter (1983) that there is a dual neuroendocrine control of the gonadotropine release hormone where there are counterbalancing stimulatory and inhibitory components. This is said to be gonadotropic release inhibitory hormone "GRIF". In addition, in fish urotensin is present and concerned with salt regulation rather than the angiotensin of mammals (Bone and Marshall, 1982). This preceded discussion is neccessary to understand some of the histological changes of the female Oreochromis niloticus gonads.

Histological changes in the fish ovaries fed on treated diets were noticed and became more apparent on going from aquarium I to IV. Thus the increase in the tendency to acquire the acidic dye followed the same trend with increase of dose of the steroid-like materials. This was more apparent in large eggs which were in various degrees of attresia, reaching the highest degree in those of aquarium IV. The noticed acidic material filled the whole ovary in those fish. This is due to the effect of estrogen which increases protein especially the acidic ones. Previous works indicated similar results on rats and chicks when treated with estrogens (White et al., 1973; Prosser, 1973; Grant, 1978 and Ganong, 1983). In those works, this was found to be caused by promotion of RNA especially that of the polyrisomes. Smaller oocytes tended to be slightly basophilic, but with acidophilic nuclei in the fish treated in aquaria I and II. The progressed attretic condition in aquarium III and finally in aquarium IV indicated that the nuclei play a major role in the effected ovaries. Concomitantly, Ganong (1983) indicated an evidence that estrogen induces changes in the cytoplasmic macromolecules; sex estroid-binding globulin SSBG; with which it combines, being neccessary for the translocation of the combined molecules to the nucleus. Consequently, the rise in the hormones or steroids will increase the SSBG level. The cell contents, accordingly would be depleted from the cytoplasmic

receptor as the complex build up in the nucleus, where the steroid and protein-like component bind to the specific chromatin sites. Increased nuclear RNA polymerase activity occurred within one hour after treatment as indicated by Babiker and Ibrahim (1979) and Ganong (1983).

Autolysis was complete in ovaries of small size fish (< 7cm). This is attributed to the stage of maturity of the fish gonad. Thus, young mature fish could not resist undergoing complex autolysis, while older fish could. This is something pertinent to a species. It is known that hormonal administration, after reaching maturity, may bring some changes to an adult but will not destroy the gonad or lead to a complete reversal (Ganong, 1983). The dissolution of the nuclear membrane in the eggs, which were undergoing autolysis, indicated that the nucleus leads this process as well. The convolution of the nuclear material and the dye phobic layer around it in those eggs, indicate that the lipid content might be in action. Steroids are lipid solluble hormones (Martin, 1979) and thus the devoid of stain area is probably due to the active transport of the lipoprotein compound.

The reduction, in the right lobe of the ovary, which is also the more effected lobe by treatment was not reported by any other similar work. However, Khallaf and Gaber (Manuscript in press) discussed the possible pathways of the steroids, and emphasized that the right gonad may receive hormonal negative feedback effect throughout extracellular fluid (Lymph) via chemical communication, and neurotransmitting inhibition in addition to the expected pathway of the feedback mechanism via anterior pituitary. This explanation, as they emphasized, still needs verification.

SUMMARY

Fish are kept in five glass aquaria, four of which are used for hormonal treatment using the pill norminest (0.5 mg norethindrone + 35 ug ethinylestradiol) in the fish diet. The dose per liter increased from aquarium I to IV by 1, 2, 3 and 4 times the original dose respectively.

An increase in the tendency to acquire the acidic dye with dose increase, and this is due to estrogen effect which increases protein synthesis especially the acidic ones. Attretic condition in the gonads; especially in small fish; progressed gradually with increase in the hormonal dose. Nuclei in ovaries with those attretic manifestations are found to play a major role in this process of attresia.

Autolysis is complete in young fish (< 7 cm in total length). In adult mature fish, the effect of treatment is partial where autolysis is not complete and developing eggs if present either are arrested or in attresia. Some ovaries may contain unidentified acidic material (Probably acidic proteins) filled most of the gonad. The right lobe of the ovary is prominently effected than the left one.

ACKNOWLEDGMENT

Thanks are due to Prof. Y.S. El-Assy; professor of comparative anatomy, Zoology Dept., Faculty of Science, Cairo University; for his kind criticism, valuable remarks and interest throughout carrying out this work.

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