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Enhancement of Reproduction in *Oreochromis niloticus* by Induction of Inhibin and Activitin-Like proteins

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ABSTRACT

The aim of the present research was to improve *Oreochromis niloticus* reproduction by treating females exogenously with L-thyroxin (T4), which will stimulate the producing of inhibin and activin A during the growth of larvae, with an influence on the growth and survival of larvae. The immunohistochemical examination was carried out on developing larvae from *O. niloticus* spawners administered T4 and control, examining the allocation of activin/inhibin subunits A and B, as well as the inhibin subunit α, from 1-28 days post-hatching. The injection of the females with T4 hormone had a notable impact on the reproductive activity, leading to an enhancement in the quality of their eggs, as demonstrated by the significantly normal larvae upon hatching. Findings from the immunohistochemical investigation showed that only inhibin/activin βA were obtained in the differentiate kidney, heart, brain, digestive system, and gills. Strong immunoreactivities of inhibin/activin βA were located in all examined tissues and ages, indicating that injections of thyroxin (1 or 5µg T4/g BW) into *O. niloticus* female significantly improved the yield of them in the growing tissues of larvae. Inhibin/activin βA could control the differentiation and growth of various organs in *O. niloticus* larvae. Consequently, T_4, inhibin, and activin A through direct or indirect means aided in the growth of *O. niloticus* larvae, as evidenced by the notable increases in larvae weight and length that obtained through the entire experiment. In addition, in comparison to the control, the treated females' larvae had a noticeably greater rate of survival. We could conclude that tilapia females' reproduction is enhanced by external T4 in the blood of females, which then transfers through the eggs to larvae. The thyroid hormone that was transferred seems to influence the production of inhibin/activin A, which could provide a unique growth advantage for the progeny of *O. niloticus*.

INTRODUCTION

Thyroid hormones (THs) have been linked to numerous biological processes, including osmoregulation, growth, morphogenesis, pigmentation of the skin, and reproduction **(Tovo-Neto *et al*., 2018; Campinho, 2019; Deal & Volkoff, 2020; Shibata *et al*., 2024; Zwahlen *et al*., 2024)**. There is proof that thyroid hormones have multiple effects on fish reproduction at various levels of the hypothalamo-hypophyseal-gonadal

axis **(Swapna & Senthilkumaran, 2007; Raine, 2011; Habibi *et al*., 2012; Tovo-Neto *et al*., 2018; Eslamizadeh *et al*., 2024)**. Thyroid hormones' function in fish growth and development in their early life was examined and reviewed **(Power *et al*., 2001; Deal & Volkoff, 2020; Shibata *et al*., 2024)**. Fish eggs and larvae are now known to contain significant levels of thyroid hormones **(Tagawa *et al*., 1994; Kang & Chang, 2004; Cabanilla-Legaspi *et al*., 2021)**. Their existence at this early phase of growth suggests that they are implicated in fish development at an early stage **(Chang *et al*., 2012; Cabanilla-Legaspi *et al*., 2021; Melianawati *et al*., 2022; Weinrauch *et al*., 2023; Liu *et al*., 2024)**. It is possible for maternal thyroid hormones to enter eggs and enhance larval development as a result **(Khalil *et al*., 2011; Brown *et al*., 2014)**. Maternal hormone administration can raise the levels of these hormones in eggs from fish **(Ayson & Lam, 1993; Kang & Chang, 2004; Brown *et al*., 2014)**.

Rearing of the Nile tilapia, *O. niloticus*, in hatcheries of Egypt has produced random and irregular fry yield. It has shown to be incredibly difficult to raise larvae., with high larval mortality happening within the weeks two and three after hatching. Taking into account that physical inability to eat following the depletion of endogenous reserves causes starvation, the reason behind these deaths could have physiological reasons. Given that T4 has a potentiating influence on fish larvae's ability to survive and grow, it was worthwhile to find out if the female brood fish injected with exogenous T4 might result in an enhanced larval growth and survival.

All vertebrates require pituitary gonadotrophins to induce oocyte maturation; however, an increasing amount of evidence also points to the importance of endogenous ovarian factors, including nonsteroidal substances (primarily activin/inhibin) **(Ge *et al*., 1992, 1997)**. The dimeric protein known as activin is a participant in the superfamily of TGF β (transforming growth factor β) **(Massagué, 1987)**. It comes in three different isoforms: activin A (βAβA), activin B (βBβB), and activin AB (βAβB). Its two b subunits are βA and βB. Despite being recognized as a protein present in ovaries at first that increases pituitary FSH (follicle-stimulating hormone) secretion **(Ling *et al*., 1986; Vale *et al*., 1986)**, it is known that activin exhibits various biological processes occurring in a range of tissues **(Vale *et al*., 1988)**. Activins and their receptors are found in numerous tissues of fish and other lower vertebrates, including mammals, where they regulate several physiological functions, such as reproduction, through both autocrine and paracrine means **(Ge *et al*., 1997; Pang & Ge, 1999; Peng & Mukai, 2000; Wu *et al*., 2000)**.

Gonadotrophin releasing hormone (GnRH) from the hypothalamus and gonadotrophins (GTHs) from the pituitary are released through the reproductive system in response to activins and inhibins **(Calogero *et al*., 1998; Peng & Mukai, 2000; Uchiyama *et al*., 2000; Bilezikjian *et al*., 2001, 2004; Aroua *et al*., 2012)**. The presence of βA and βB subunits immunoreactivities in the ovary, testis, brain, and pituitary of fish indicates that activin plays endocrine, paracrine, and autocrine functions in controlling

fish reproduction **(Ge *et al*., 1997; Mousa & Mousa, 2003; Petrino *et al*., 2007; Ahmad *et al*., 2018, 2020)**. Additionally, activin/inhibin βA has been demonstrated in fish ovaries and could be crucial for fish reproduction during oocyte development and maturation **(Garg & Peng, 1998; Wu *et al*., 2000; Mousa & Mousa, 2003; Ahmad *et al*., 2020; Zhao *et al*., 2022)**. Moreover, activin B appears to be required for fostering the spermatogenesis of *Anguilla japonica*, the Japanese eel, *in vitro* as well as *in vivo* **(LaPolt *et al*., 1990; Miura *et al*., 1995)**.

Despite evidence of activin in mature fish species, few details are available regarding the localization and classification of activin in fish larvae during early phases of both normal and induced development. The study's objective was to investigate how *O. niloticus* reproductive performance was influenced by maternal injections of T4. Furthermore, we reported on the immunocytochemical localization of activin in *O. niloticus* larvae that were taken from T4-injected and control females, as well as the subsequent impact on the growth and survival of larvae.

MATERIALS AND METHODS

Research location

This investigation was completed from January 1, 2023, to July 30, 2023, at El-Serw Station of Fish Research.

Spawning and treatments

In spawning hapas, semi-natural spawning occurred on May 1st (temperature: 23–25°C), according to recent report by **Khalil *et al*. (2024)**. Three groups of three treatments, each with ten duplicates, were randomly assigned to female participants. T4 (Sigma Chem. Co.) with DMSO (dimethylsulfoxide) as a solvent was injected once into fish at dosages of 1 and 5µg T4 for every gram of fish, in treatment 1 and 2. Treatment 3 fish as control received only DMSO injections. Females were permitted to spawn after injection and were being stocked with running males that were not hormone-treated. The breeding activity was observed every day. After mating, the laying female was left to incubate the young. For every female, the ovulated eggs number and the average diameter were recorded. The following rates were found for each female that spawned:

Fertilization rate (%) = 100 X no. of developed eggs /no. of spawned eggs.

Hatching rate (%) = 100 X no. of hatched larvae/no. of fertilized eggs.

Normal hatched larvae (%) = 100 X no. of normal hatched larvae/no. of hatched larvae.

Larvae obtaining and analyzing

20 larvae were sampled at random every week (five times) for 5 weeks of age. The larvae underwent anesthesia, and every individual was separately weighed and measured. The number of larvae that survived, as well as the overall average weight and length were determined for each therapy at the trial end.

The samples were fixed *in toto* in Bouin's fluid at an ambient temperature for two days after undergoing anesthesia in a solution of clove oil at a level of 40mg/ l for histological

examination. The samples were transferred to 70% ethyl alcohol after fixation and dehydrated in different grades of ethanol. After xylene clearing, paraplast (M.P. 56–58˚C) was used to embed the samples. Then, 5µm-thick serial sections were cut and mounted on glass slides.

Immunocytochemical procedures

Antibodies

Dr. W. Vale (The Salk Institute, La Jolla, CA) provided rabbit anti-porcine inhibin α (1-26)-Gly-Tyr (Code 140-249-1), anti-cyclic inhibin βA (81-113)-NH2 (Code 305-24-D), and anti-cyclic inhibin βB (80-112)-NH2 (Code 305-25-D).

Immunocytochemical reactions

As previously mentioned **(Mousa & Mousa, 1999)**, immunocytochemical staining was typically carried out using an ABC (Avidin-biotin peroxidase complex) Kit. Overnight at 4°C, sections were kept in primary antibodies diluted at 1:1000 for anti-α and 1:2000 for anti-βA and anti-βB against subunits of activin and inhibin. Following that, the slides were finished according to **Mousa and Mousa (1999)**.

Statistical analysis

The of SPSS program (Statistical Package for Social Sciences) was utilized to examine the data. The paired samples "t" test was compared using means. A statistically significant result was defined as $P< 0.05$.

RESULTS

I. The effects of hormone therapy on reproductive activity

In comparison to control, a high percentage (80– 100) of the tilapia broodstock was injected with T4 spawned earlier, at intervals ranging from 5 to 12 days after injection (Table 1). A notable variation existed in the number of ovulated eggs between the females who received T4 injection and the control. Furthermore, as demonstrated by Table (1), the spawned eggs number increased significantly more when treated with T4 high dose than when treated with low dose. There were noticeable alterations in the eggs' diameters. In comparison to control, the T4-injected group possessed a greater quantity of larger eggs.

In general, eggs from females injected with T4 high dose had a higher fertilizability than the eggs in control and from females injected with a low dose (Table 1). In contrast to control, the T4-injected groups' eggs possessed a greater hatchability rate. In the T4-treated groups, an elevated proportion (85– 94) of newly hatched larvae are normal, as depicted in Table (1).

Table (1) shows that injecting female *O. niloticus* with T4 improved larval survival, as indicated by the reduced mortality rates observed during the 35-day rearing period.

Compared to the group under control, the T4-treated female larvae showed a noticeably greater rate of survival. Furthermore, in contrast to low dose injections, females receiving thyroxin high doses had a noticeably greater rate of survival. The larvae from T4-treated females also demonstrated noticeably higher average body weight and length in contrast to the control, as indicated in Table (1), indicating that the hormonal treatment also enhanced the growth of larvae.

II. The influence of hormone therapy on the immunoreactivity of inhibin and activin βA

Immunohistochemical investigation was conducted on *O. niloticus* larvae addressing the development from spawners treated with T4 and control, observing the localization of subunits βA and βB of inhibin and activin, as well as inhibin subunit α, from 1 to 28 days post-fertilization. The data of the immunohistochemical study indicated that only inhibin and activin βA were discovered in the growing kidney, heart, brain, digestive tract, and gills. T_4 therapy for *O. niloticus* females significantly enhanced the immunoreaction of inhibin/activin βA within the developing larvae's tissues.

Gills: The chloride-secreting cells in gill filaments were the primary sites of immunostaining for inhibin and activin βA (Fig. 1). Chloride cell immunoreactivities increased as the larva developed, as demonstrated by a rise in chloride cell number and immunoreactivity at 21 dph (Fig. 1e, f). The high dose of T4 (5μg T4/g BW) injected into female larvae improved the synthesis of inhibin/activin βA in their developing gills. This was demonstrated by the increased number and strong immunoreactivities of chloride cells in all investigated ages (Fig. 1b, d, f).

Digestive system: As stated by the immunocytochemical analysis, the intestine and stomach are two distinct areas of the developing digestive tract where inhibin and activin βA are localized (Figs. 2, 3). Furthermore, it was noted that the liver and pancreas, two accessory glands, exhibited immunoreactivities to inhibin and activin βA (Figs. 2, 3).

The mucosal epithelium and the stomach gastric glands were the only areas where inhibin and activin βA immunoreactivity was found (Figs. 2c, d, 3a, b, e, f). The stomach showed weak activin βA and inhibin immunoreactivity at 21 dph, (Fig. 3a). But as the larva developed, its immunoreactivity rose in the stomach and at 28 days post-hatching, it demonstrated strong immunoreactivity (Fig. 3e, f). Figs. (2d, 3b f) show that treating *O. niloticus* females with T_4 significantly increased the inhibin/activin βA synthesis in the growing larval stomach.

During the control larvae development, very weak inhibin/activin βA immunoreactivity was observed in the submucosal and mucosal layers in the intestine (Figs. 2a, e, 3c). However, in the layers of mucosal and submucosal of the larvae from the T_4-treated female group, there was an intense immunoreaction to inhibin and activin βA, especially in the developmental early stages at 7 dph (Fig. 2b). Furthermore, treating female *O. niloticus* with T_4 significantly increased the liver and pancreas production of

inhibin/activin βA in the growing larvae, which was extremely low within the group under control (Figs. 2f, 3d).

Other organs: Furthermore, immunoreactivity for inhibin and activin βA was found not only in the digestive system and gills but also in the heart, brain, and kidney (Figs. 4, 5). Immunoreactivity to inhibin and activin βA was discovered in the cardiac muscle. As seen in Fig. (4), the ventricle muscle in the heart of larvae from T_4-treated females group showed a stronger immunoreaction with inhibin and activin βA antibody than that acquired from the group of the control. Furthermore, the organum vasculosum laminae terminalis of the midbrain (Fig. 5a, b) and the kidney's urinary tubules (Fig. 5c–f) both showed inhibin and activin βA immunoreactivity. The larvae from T_4-treated female group showed an increased immunoreactivity of inhibin and activin βA in these organs (Fig. 5b, d, f).

Table 1. Effect of exogenous thyroxine injection on the reproductive activity of *O. niloticus* females

Reproductive activity	Control	Treated	
		T_1 (1 µg T_4 /gm BW)	**T_2** (5 µg T_4 /gm BW)
Spawning activity:			
Time of spawning (days after injection)	15-20	10-12	5-7
% of spawned females	40	80	100
Number of Ovulated eggs/female	650±50	960±75 [a]	1450±120 [b]
Mean of ovulated eggs diameter	1.25±0.10	1.54±0.05 [a]	1.72±0.04 [b]
Fertilization rate %	40±2.40	70±3.30 [a]	85±6.40 [b]
Hatching rate %	50±3.33	75±3.30 [a]	90±5.50 [b]
Normal hatched larvae %	60±3.83	85±3.03 [a]	94±2.65 [b]
Larval production (after 35 dph):			
Survived larvae %	40±1.43	76±1.11 [a]	90.4±1.1 [b]
Final larval length (mm)	32.0±1.62	36.0±1.52 [a]	40.0±1.34 [b]
Final larval weight (mg)	530±26.56	720 ±47.46 [a]	950 ±68.21 [b]

a, b: Significant differences when compared to control (P<0.005).
All mean values are presented ±SD.

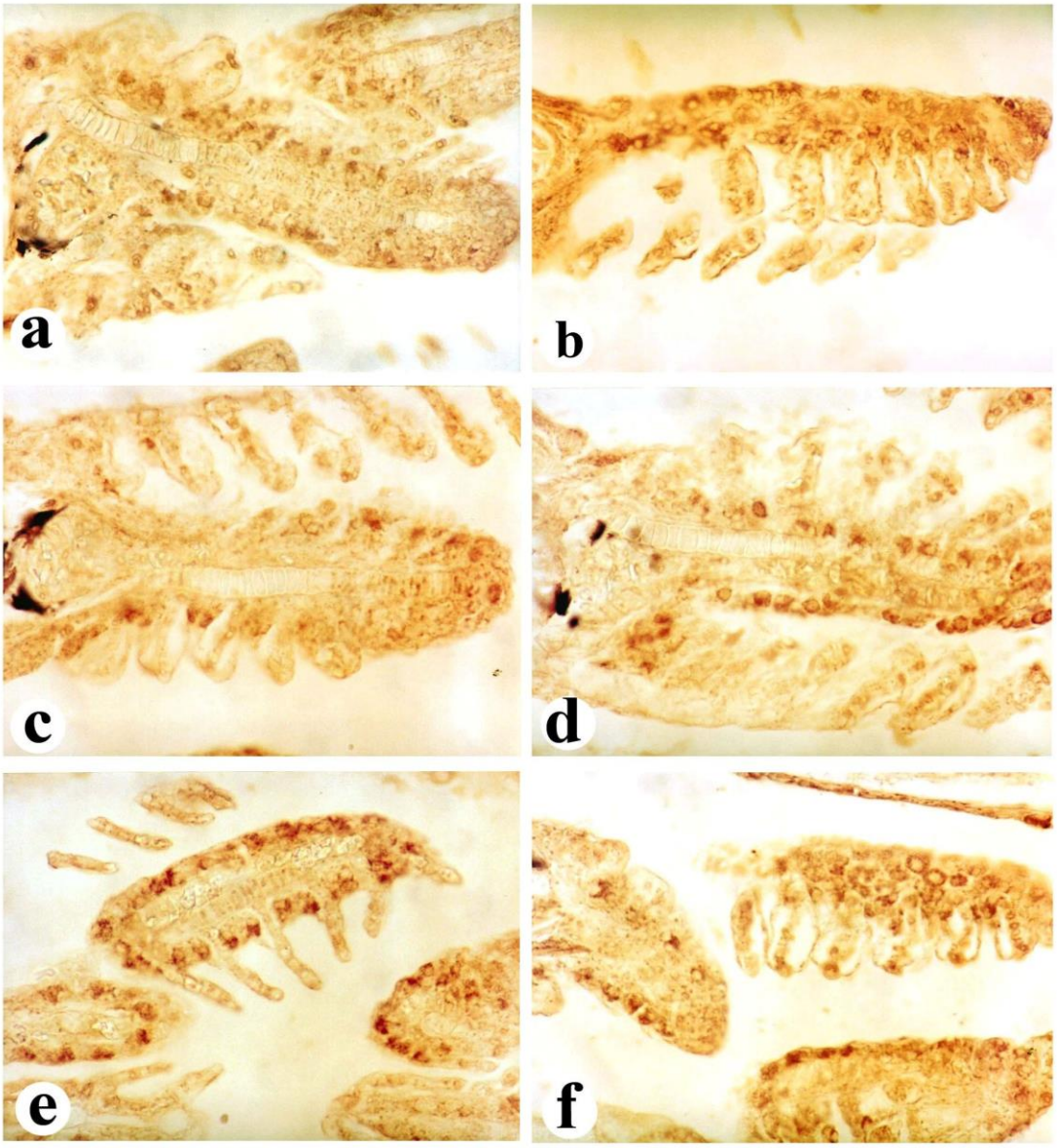

Fig. 1. *O. niloticus* larvae gill sections immunostained with rabbit cyclic inhibin and activin βA antiserum. The cells secreting chloride in the gill filament are the primary locations of immunostaining. X200. a) 7 dph larva from control group. b) 7 dph larva from treated group. c) 14 dph larva from control group. d) 14 dph larva from treated group. e) 21 dph larva from control group. f) 21 dph larva from treated group. Strong activin βA immunoreactivity was seen in the treated group

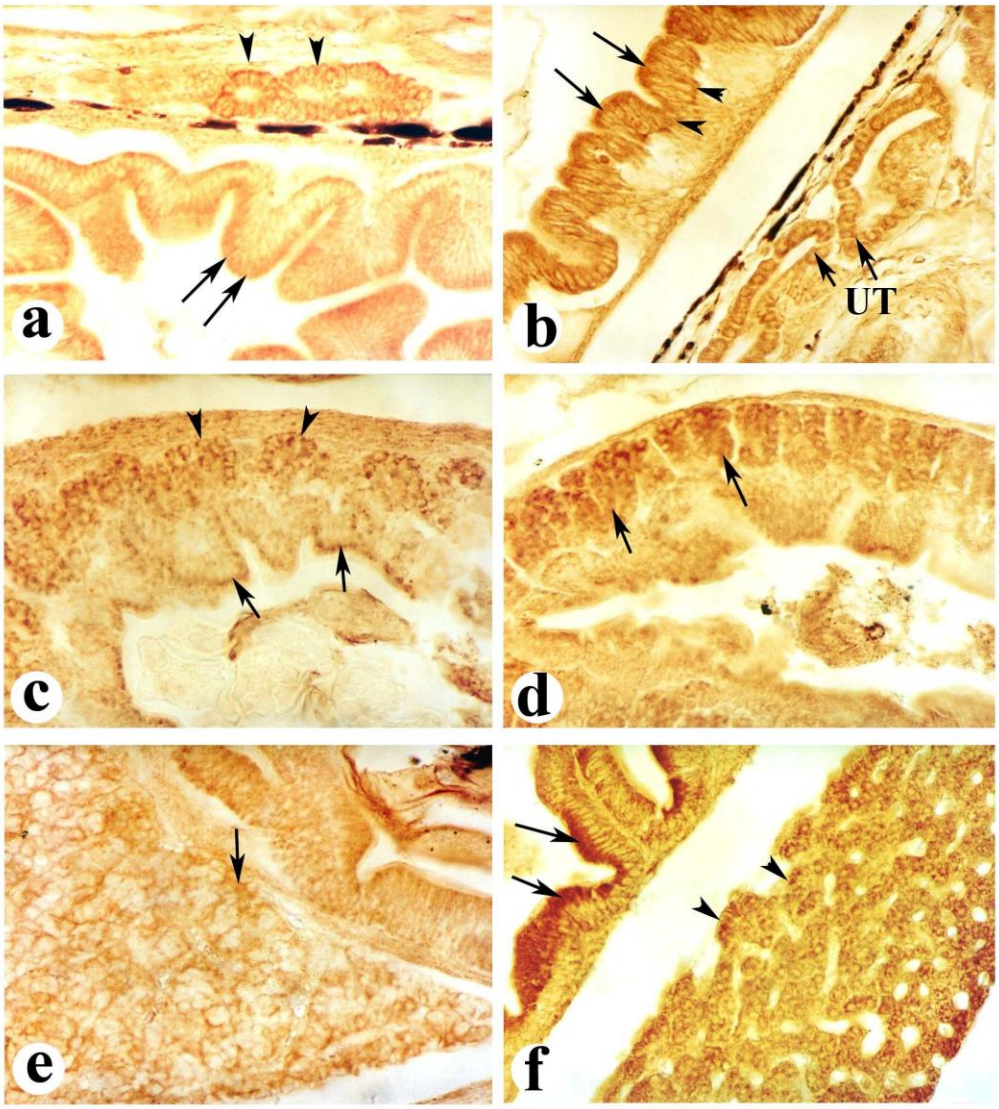

Fig. 2. Rabbit antibodies against the βA subunit of cyclic inhibin and activin were used to immunostain the sagittal sections of *O. niloticus* larvae. X200. **a)** Seven days after hatching, the gut of the control group's larvae exhibits both a weak mucosal layer immunoreactivity (arrows) and a positive urinary tubule immunoreactivity (arrowheads). **b)** Strong immunoreactivity in the mucosal (arrows) and submucosal layers (arrowheads) of the larvae's gut at 7 dph from the treated group. In the urinary tubules (UT), a strong immunoreaction is also seen. **c)** Twenty-one days after hatching, the larvae stomach of the group of control indicated moderate immunoreactivity in the gastric glands (arrowheads) and mild immunoreactivity in the mucosal layer (arrows). **d)** The larvae's stomach of the treated group at 21 days post-hatching displays prominent immunoreactivity in the gastric glands (arrows). **e)** A weak liver immunoreaction (arrow) was found 21 days after hatching in the control group larvae's gut, along with a negative immune response. **f)** Larvae's gut from the treated group at 21 days post-harvest displaying strong immunoreactivity in the mucosal layer (arrows) and moderate liver immunoreactivity (arrowheads)

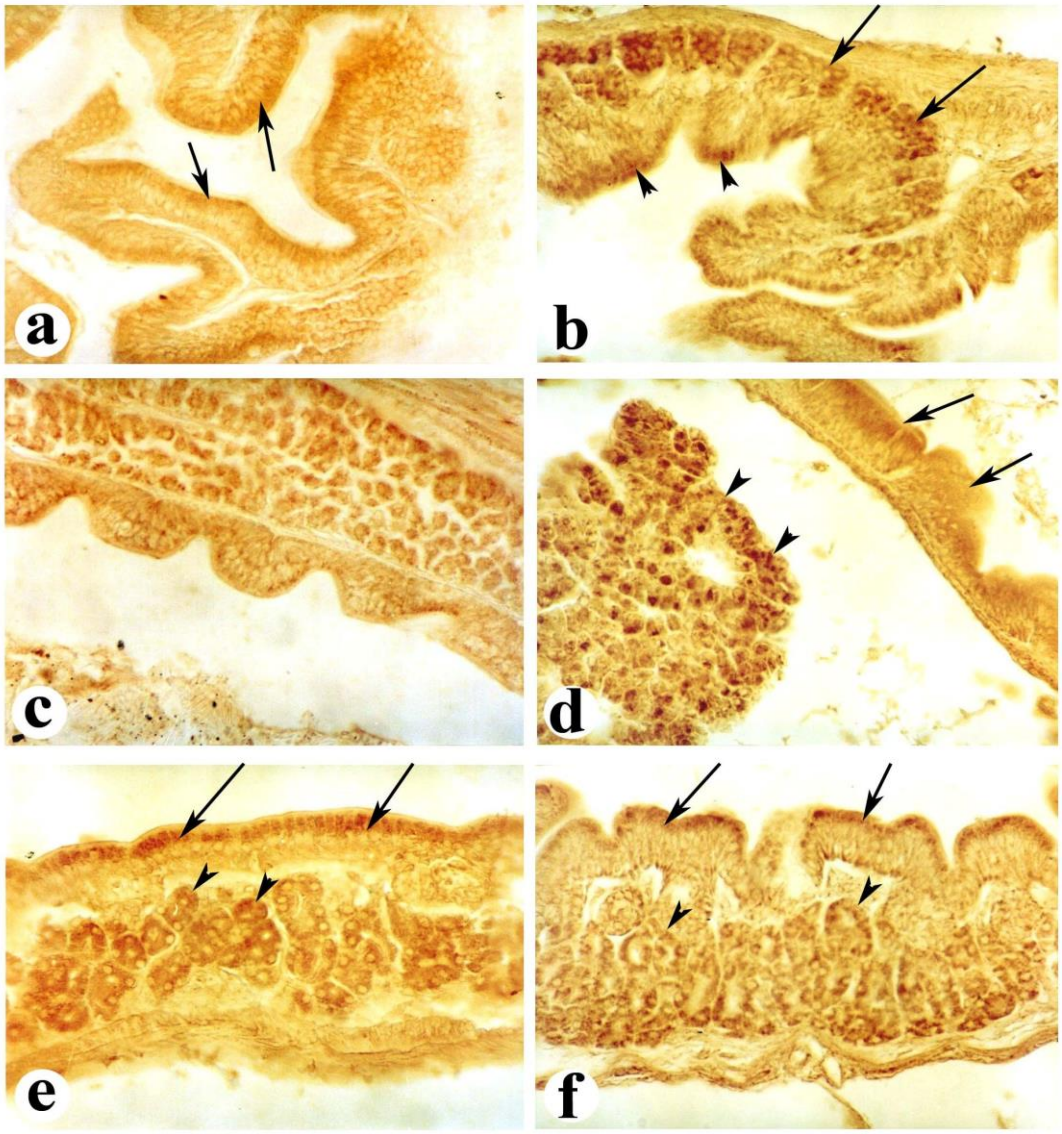

Fig. 3. Rabbit antibodies against cyclic inhibin and activin βA subunit were used to immunostain the sagittal sections of *O. niloticus* larvae. X200. **a)** A larva from the control group had weak immunoreactivity in its stomach mucosal layer at 21 days post-hatching (arrows). **b)** At 21 days after hatching, the treated larvae' stomach showed moderate immunoreaction in the layer of mucosa (arrowheads) and strong immunoreactivity in the gastric glands (arrows). **c)** Control larvae showing weak immunoreaction in their pancreas and intestine at 21 dph. **d)** The treated larvae intestine showed signs of moderate immunostaining in the submucosal layer (arrows) and intense immunoreaction in the pancreas (arrowheads) at 21 dph. **e)** The control group's stomach at 28 days post-hatching demonstrates a strong immunoreaction in the gastric glands (arrowheads) and mucosal layer (arrows). X400. **f)** Strong immunoreactivity was seen in the mucosal layer (arrows) and gastric glands (arrowheads) of the larvae stomach from the treated group at 28 dph. X400

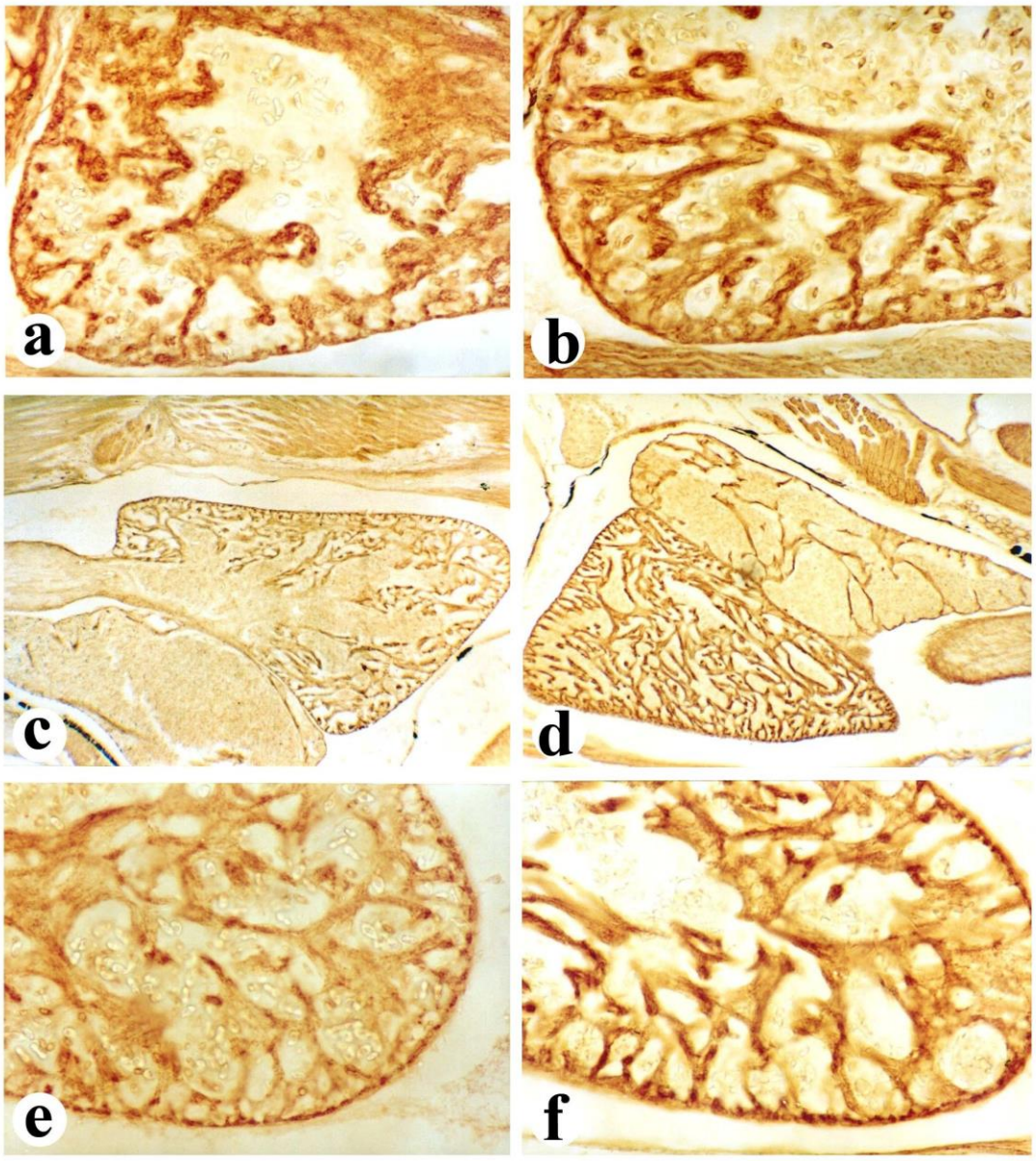

Fig. 4. Heart sections from *O. niloticus* larvae, displaying the ventricle's cardiac muscle, immunostained with rabbit antibodies against cyclic inhibin and activin βA. **(a, b, e and f)** X200, **(c and d)** X100. **a)** 7 dph larvae belonging to the control group. **b)** 7 dph larva from treated group. **c)** 21 dph control larva . **d)** Larvae from the treated group, 21 days old. **e)** Magnification of **(c)**. **f)** Magnification of **(d)**. Note, the strong inhibin and activin βA immunoreactivity in the treated group

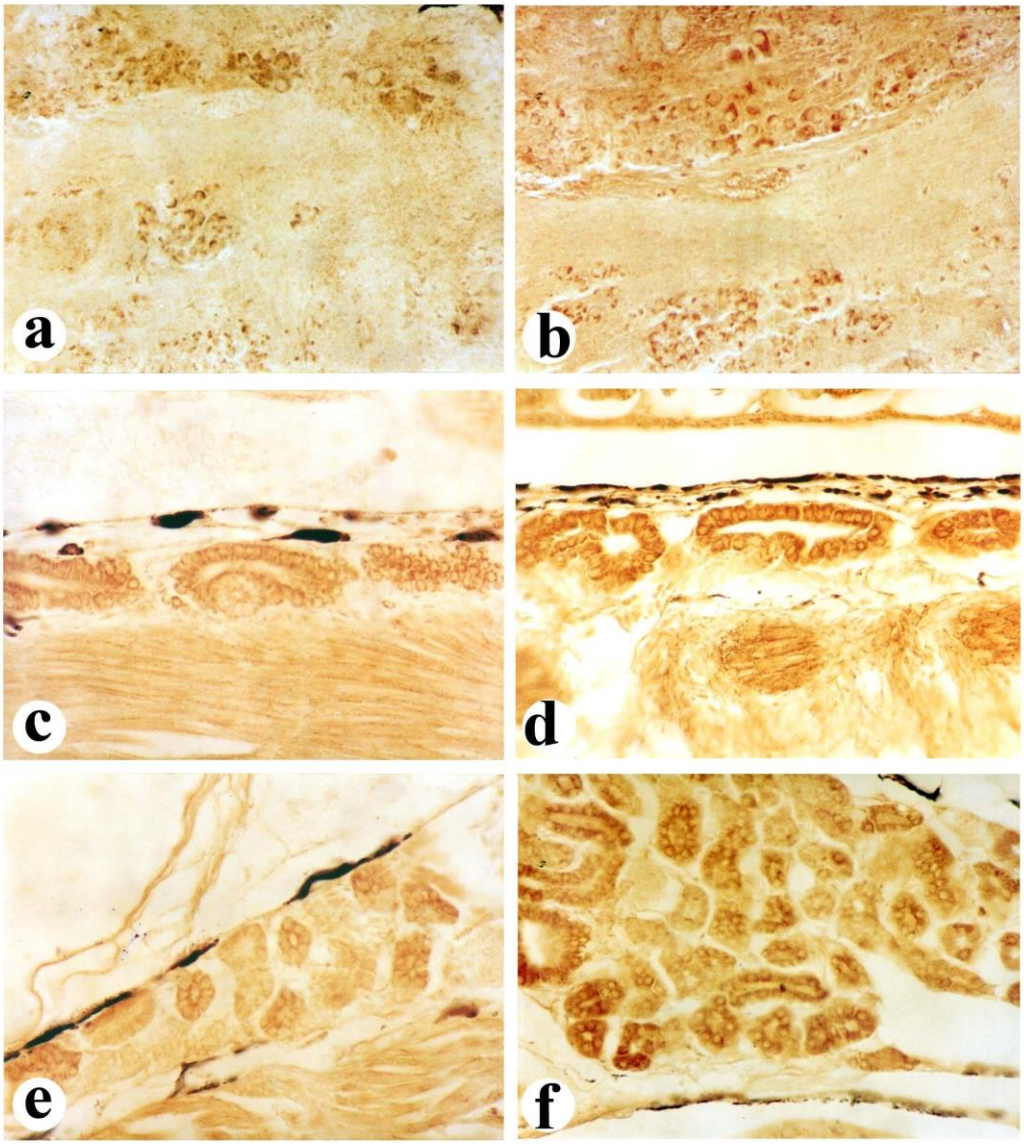

Fig. 5. Sections sagittally in *O. niloticus* larvae, represented different organs, immunoreacted with rabbit antibodies against inhibin and activin βA subunit. X200. **a)** Brain of 7 dph larva from control group. **b)** Brain of 7 dph larva from treated group. **(c-f)** Parts of urinary tubules of kidney. **c)** 7 dph larva from control group. **d)** 7 dph larva from treated group. **e)** 21 dph larva from control group. **f)** 21 dph larva from treated group. Note, the strong inhibin and activin βA-immunoreactivity in the treated larvae different organs at all investigated ages

DISCUSSION

Thyroid hormones regulate the evolution and metabolism of ovarian tissues, making them essential for the healthy function of the female reproductive system. By increasing the quantity of spawned eggs, the diameter of eggs, the rate of fertilization, and the rate of hatching, thyroxin supplementation in *O. niloticus* has a great chance to enhance the ability of female broodstock to reproduce. The injection of T4 (1 or 5 μg T4/g BW) hormone into the mature females had a noteworthy impact on their reproductive performance, resulting in an enhanced egg quality, as indicated by the remarkably normal-looking larvae after hatching. During the reproduction of fish, the activin-inhibin system is necessary for folliculogenesis and ovarian homeostasis in particular **(Ahmad *et al*., 2020; Zhao *et al*., 2022)**. Maternal T4 can influence ovary maturation either through direct or indirect influence of the production of inhibin and activin, which can control gonadotropin hormones at the hypophysis directly or indirectly by influencing the manifestation of the GnRHR gene and the discharge of GnRH **(Gregory & Kaiser, 2004; Bilezikjian *et al*., 2004, 2006; Aroua *et al*., 2012)**. In this regard, **Mousa and Mousa (2003)** reported that the *L. ramada* ovary produces inhibin and activin, which may be released during vitellogenesis. These substances are then carried to the pituitary, where they cause the production of additional GTH, which is necessary for vitellogenesis and oocyte growth. Moreover, subunits of activin and inhibin, which are regional controllers in teleost gonads and induced gonad maturation, were shown to be present in *F. heteroclitus* ovary **(Petrino *et al*., 2007)** and the gonads of *Labeo rohita* **(Patnaik *et al*., 2021)**. Similarly, thyroxin supplementation foe the African catfish resulted in higher vitellogenin concentrations and spawning egg diameters, which hold a significant promise for enhancing the reproductive efficiency **(Rawung *et al*., 2020)**. Additionally, the growth and acceleration of gonadal maturation in *S. serrata* were greatly affected by the application of thyroxin hormone as an ovary maturation stimulant **(Iromo *et al*., 2015, 2018, 2021)**. Thyroid hormones and cortisol from the mother are said to be placed within the yolk of fish eggs, speeding up the differentiation of the larvae's organ systems until they are able to perform endogenous endocrine functions **(Brown *et al*., 2014)**.

Furthermore, this study examined how exogenous T4 treatment of *O. niloticus* females affects the immunoreactivity of inhibin and activin βA during larval development, and how this affects larval growth and survival. According to the immunohistochemical analysis, only inhibin and activin βA were found in the developing gills, digestive system, heart, brain, and kidney of *O. niloticus* larvae. The immunoreactivities of these organs to inhibin and activin βA were enhanced during larval development. A constituent of the superfamily known as transforming growth factor-beta, or TGF beta with a broad anatomical distribution, inhibin and activin βA may control the growth and distinction of various organs in *O. niloticus* larvae. The administration of thyroxin (1 or 5μg T4/g BW) into *O. niloticus* females significantly increased the

quantity of inhibin and activin βA generated in the growing tissues of larvae, as evidenced by the strong immunoreactivities among the two proteins identified in all examined tissues and ages. The synthesis of inhibin/activin βA is possibly directly impacted by exogenous thyroxin, which enters oocytes and larvae from the mother's circulation. Comparable findings were observed in the *Siganus guttatus* rabbitfish. **(Ayson & Lam, 1993)** and the *Sebastes schlegeli* rockfish **(Kang & Chang, 2004)**. Therefore, since there was a greater growth in the larvae's weight and length throughout the experiment, both thyroxin and inhibin/activin βA either directly or indirectly enhanced the larval growth of *O. niloticus*. In comparison to the group of control, the treated females' larvae exhibited a markedly increased survival rate. Similar studies demonstrated that hormones of the thyroid increase the teleost larvae's chances of survival and quicken their development and progress **(Brown & Kim, 1995; Ansal & Kaur, 1998; Power *et al*., 2001; Gavlik *et al*., 2002; Cabanilla-Legaspi *et al*., 2021)**.

The tilapia larvae respond in a manner dependent on the dosage of thyroxin therapy. Weight, length, and percentage of the survival of the tilapia larvae from group treated with 5μg T4/g BW were noticeably greater than those treated with 1μg T4/g BW. Similar findings were noted for the *Siganus guttatus* **(Ayson & Lam, 1993)**, *Epinephelus coioides* **(de Jesus *et al*., 1998)**, and *Anabas testudineus* **(Alang *et al*., 2020)**.

Thyroid hormones have been demonstrated to either directly or indirectly increase food utilization and/or appetite by inducing growth hormone secretion. This, in turn, raises food conversion efficiency, as evidenced by lower food conversion ratio values **(Fagerlund *et al*., 1984; Higgs *et al*., 1992; Ansal & Kaur, 1998)**. Additionally, thyroxin treatment speeds up the progress of the stomach organs in the early metamorphosis stages of *Paralychthys dentatus*. Stomach development is delayed by thiourea (Tu)-induced inhibition of T4 synthesis **(Soffientino & Specke, 2000)**. As stated by the current study, T4-induced increases in inhibin and activin βA release in the digestive system may improve food utilization during the critical time of first feeding, as well as accelerating growth and reducing the death rate. Adminstering thyroid hormones to marine fish larvae was beneficial since T4 seems to raise the acceptance of proteins and fats in the digestive tract **(Tanaka *et al*., 1995)**. Furthermore, **Woo *et al*. (1991)** found that oral delivery of T3 resulted in higher intestinal enzyme activity, which in turn increased appetite and effectiveness of food conversion in under yearling *Chrysophyrys major*. Additional reports of an improved ratio of food conversion in *Channa striatus* **(Arul, 1987)** and *C. carpio* **(Kumar *et al*., 1991)** has been produced after T4 administration. Oral T3 administration is recognized to improve the effectiveness of food conversion in the salmonids **(Fagerlund *et al*., 1984; Higgs *et al*., 1992)**.

In conclusion, the ability of *O. niloticus* females to reproduce was enhanced by the external T4 in the bloodstream of females at the time inserted into the eggs and larvae. The thyroid hormone transfer offers a transparent benefit for the development of

progeny and seems to be involved, either through direct or indirect means, in the larvae's early development through the output of inhibin and activin βA.

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Conflict of interest

The authors state that there were no conflicts of interest.

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