



Effect of Introducing Foreign DNA into Nile Tilapia, *Oreochromis Niloticus* Gonads on Male Fertility of the Progeny Produced Under Salinity Stress

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Research Article

Volume 6 Issue 2

Received Date: October 21, 2021

Published Date: November 10, 2021

DOI: 10.23880/oajvsr-16000216

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Abstract

This work was conducted to study the effect of transfer foreign DNA isolated from sea bream, *Sparus aurata* or Artemia, *Artemia salina* into the ovaries and testes of *O. niloticus* adult on male fertility of the progeny produced under different salinity levels through studying the histological changes of testes. The results showed that male fertility of *O. niloticus* decreased as a result of introducing foreign DNA into the gonads of their parents. This decrease depends on the type of DNA since the results of the testes investigation revealed that *O. niloticus* received Artemia DNA displayed normal lobules of 40 %. In contrast, *O. niloticus* received sea bream DNA, showed only 28 % of normal lobules. The results also showed that the fertility of males decreased with increasing salinity levels up to 36 ppt. Although all fish received foreign DNA displayed low fertility, Nile tilapia received Artemia DNA showed more salinity tolerance and highest fertility than Nile tilapia received sea bream DNA.

Keywords: Nile Tilapia; *Oreochromis Niloticus* Gonads; Progeny; Salinity Stress

Introduction

The shortage in freshwater in many countries and its increasing demands in agriculture and other urban activities has increased the pressure to develop aquaculture in brackish water and seawater [1]. Tilapia are important species, especially for tropical aquaculture and euryhaline fish, that can live and thrive in a wide range of salinity from freshwater to full seawater even though some species tolerate a wider

salinity range than others [2,3]. Tilapia (including all species) is the second most important group of farmed fish after carps and the most widely grown of any farmed fish. In 2004 tilapia moved up to the eighth most popular seafood in the USA global production of all species of tilapia, and is projected to increase from 1.5 million tons in 2003 to 2.5 million tons by 2010, with a sales value of more than USD 5 billion. Most of this enhanced production is expected to be attributed to Nile tilapia [4] A major goal in introducing new genetic

materials into the fish genome is to establish new, improved commercial strains in aquaculture [5,6]. Genetically modified fish offer new potential for increased production of cultured organisms. This technology allows the introduction of new traits, or improvement of old ones, in a way that is impossible to be achieved with conventional breeding methods [7]. Many species of genetically modified fish have been developed since the first genetically modified goldfish was reported Aleström P, et al. [8]. The production of genetically modified fish has become a popular technique not only for producing desirable traits but also for studying mechanisms of developmental regulation of various genes and gene promoters like faster growth rate, disease resistance, cold or salinity tolerance [5,6]. Therefore, the objective of this work was to study the effect of transfer foreign DNA isolated from sea bream, *Sparus aurata* or Artemia, *Artemia salina* into the ovaries and testes of *O. niloticus* adult on males fertility of the progeny produced under different salinity levels through study the histological changes of testes and Microsatellite DNA (ISSR) analysis.

Materials and Methods

The experimental work was undertaken at Fish farm and the laboratory of Breeding and Production of Fish, Animal and Fish Production Department, Faculty of Agriculture (Saba- Basha), Alexandria University, Alexandria, Egypt.

Fish Origin

The Nile tilapia used in this study descended from a randomly mating population at the private Fish Farm, El-Behera Governorate, Egypt.

Experimental Design

Preparation of Genomic DNA: A high molecular weight DNA was isolated according to the [9] method by reducing liver sample from sea bream, *S. aurata* and whole tissue sample of Artemia, *A. salina*. The extracted DNA was restricted by the Eco R1 restriction enzyme type II. It digested DNA between guanine and adenine [10]. Then, a 20 µg/0.1 ml/fish concentration was adjusted by extrapolating the dilutions for each type of DNA extracted using 0.1 x SSC buffers [11].

Injection of Genomic DNA into Fish Gonads: Adult Nile tilapia, *O. niloticus* with an average live weight (63.00 ± 1.30 g/male and 55.50 ± 1.50 g/female), were chosen. The readiness of females to spawn was ascertained by examining the degree of swelling of the urogenital papilla [12]. Males were examined by stripping sperm [13]. Two males and four females were injected directly with the foreign DNA into fish gonads using a hypodermic needle; besides, the control group

(two males and four females) was carried out. To inoculate the adult fish, the needle was inserted into the oviduct and sperm duct [11,14]. Immediately after DNA treatments were carried out, each treated fish was stocked separately in fibreglass tanks (total volume 350 litre) supplied with dechlorinated water with adequate aeration. Brood fish were fed twice daily pellet containing 26% protein up to satiation for six days a week.

Base Generation (F0)

Culture Conditions: Base generation (F0) offspring produced from genetically modified Nile tilapia and their control were collected, counted and weighed. Fry was transferred separately to glass aquaria (100 x 34 x 50 cm) at a density of 1 fish/10 L and randomly divided for subsequent different salinity treatments. The glass aquaria were supplied with fresh dechlorinated tap water and aeration. Water temperatures were maintained at 28.00°C.

Saline Water Acclimation: Two salinity levels (18 and 36 ppt) were prepared by mixing freshwater with crude natural salt [5] obtained from El-Nasr Company for salt, Borg El-Arab, Alexandria, Egypt, beside the third group of freshwater used as control. Fry obtained from each treatment of DNA, and their control was gradually acclimated to the respective salinities by raising the salinity at the rate of 4 ppt daily [15]. Moreover, the fourth group of each treatment of DNA and their control were transferred directly to 16 ppt. Water in each glass aquaria was partially changed once daily and every three days. Fries were fed three times daily with pellets containing 38% protein, to satiation, six days a week for 70 days. Then, all the fish were fed on a diet containing 32% to satiation, six days a week up to the end of the experiment (90 days). Fish were weighed and counted biweekly. A refractometer (S/Mill-E, ATAGO Co., LTD) was used to measure salinity.

Histological Characteristics of Gonads

By the end of the experiment, testes of ten males from each treatment were carefully removed and separately fixed in 10% formal saline solution. The fixed testes were washed in 70% ethyl alcohol for three days before dehydration, then cleared and embedded in paraffin wax for the histological study. Sections of 6-10 µm thick were stained with Eirlich hematoxylin and Eosin (H&E).

Microsatellite DNA (ISSR) Analysis

DNA was extracted from the muscle, liver and gonad tissues of the base generation (F0) of genetically modified Nile tilapia injected with Artemia DNA. In contrast, their

control DNA was extracted from liver tissue only, following the method described by [9]. In this work, five base long oligonucleotide primers (Table 1) were used to initiate polymerase chain reaction (PCR) amplification. Primers were randomly selected based on GC content and annealing temperature for amplification. The PCR amplifications were performed following the procedure of [16,17]. The reaction (25 μ l) was carried out using 0.8 U of Taq DNA polymerase (Fanzyme), 25 pmol dNTPs and 25 pmol of random primer, 2.5 μ l. 10X TaqDNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (Eppendorf®). The PCR programme included an initial denaturation step at 94°C for 2 min followed by 45 cycles with 94°C for 30 s. For DNA denaturation, the annealing temperature of each primer, as indicated in Table 1, extends at 72°C for 30 s and finally extends at 72°C for 10 min. The samples were cooled at 4°C.

The amplified DNA fragments were separated on 1.5% agarose gel and stained with ethidium bromide. DNA marker (bp1000, 900 to 200) was used in this study. Moreover, to ensure that the amplified DNA bands originated from genomic DNA and not from primer artefacts, negative control (without DNA source) was carried out for each primer.

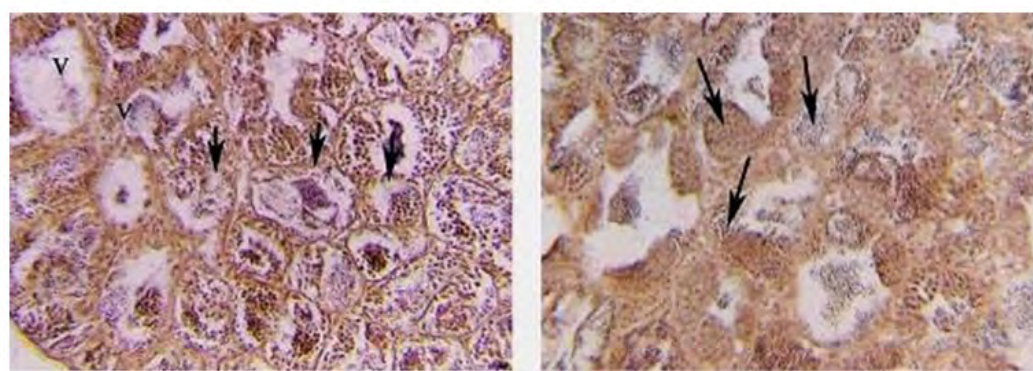
Primers code	Sequences 5`- 3`
1	GAGAGAGAGAGAGAGAT
2	CACACACACACAT
3	TATATATATATATATATAG
4	AGAGAGAGAGAGAGAGTG
5	AGAGAGAGAGAGAGAGTC

Table 1: The sequences of the different primers used.

Results

Histopathological Findings

The ripe testes of *O. niloticus* control male reared at freshwater were composed mainly of nests or lobules separated by interlobular tissue and showed more active spermatogenesis at all stages of development. Spermatozoa fill the lobular lumen (Figures 1a,b). The interlobular connective tissue is relatively thin (6 mm in thickness) and rich in interstitial cells and blood capillaries. Also, the results showed marked asynchronous development in spermatogenesis during the annual reproductive cycle (Figure 1b). Treatment combination. The amplified pattern was visualized on an UV trans illuminator and photographed by a Gel Documentation system.



1a

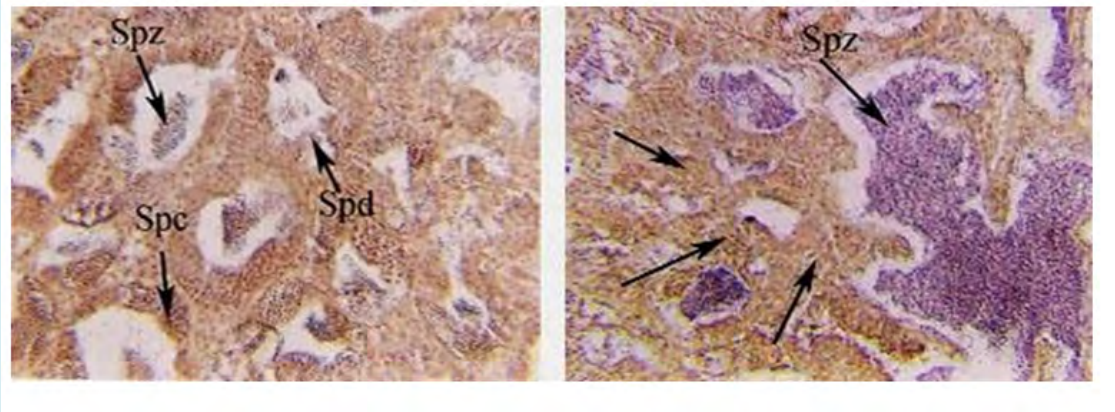
1b

Figure 1a: Photomicrograph of cross-section (CS) in the testes of the control group of *O. niloticus* reared at freshwater, showing different stages of spermatogenesis within the sharp boundary somniferous lobules (arrows), vacuoles (v). (H and E) X 250.

Figure 1b: Magnified section of the figure (1a) showing different stages of spermatogenesis (arrows). (H and E) X400.

Cross-section of testes of *O. niloticus* control male reared at 18 ppt of salinity showed that the percentage of normal lobules was 70% from total cells. In the cross-section of abnormal testes, spermatozoa were detected with few numbers of spermatids. The sections were characterized by the presence of discharge sperms and spaces free from

spermatogenesis as indicated in (Figure 2a, 2b). The testes of *O. niloticus* control male, reared at 18 ppt of salinity, was considered as more or less similar to normal tilapia; the percentage of similarity was about 70%. The interlobular connective tissue was normal in shape, except some tubules ruptures and disintegrated to form large tubules (Figure 2b).



2 a

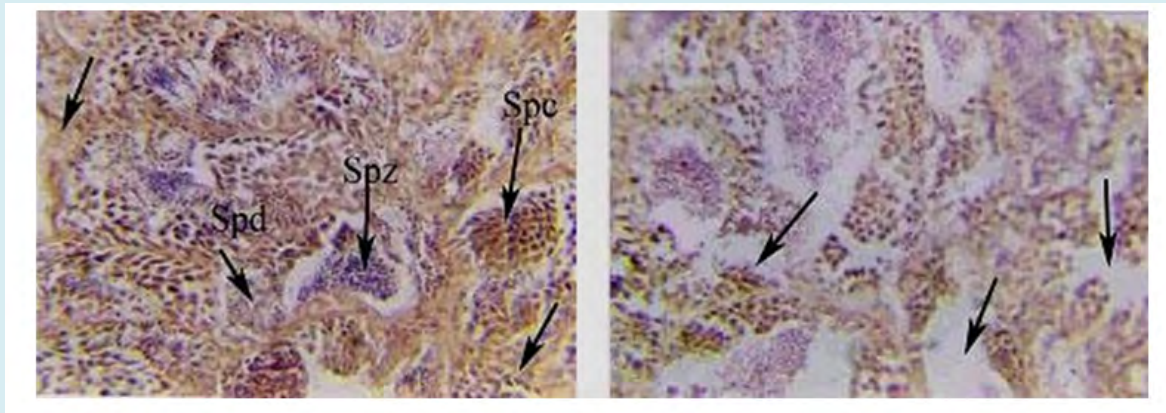
2 b

Figure 2a: Photomicrograph of cross-section (CS) in the testes of the control group of *O. niloticus* reared at 18 ppt of salinity, showing a different stage of spermatogenesis (spermatocyte (spc) and spermatid (spd)) and a moderate number of trapped sperms (spz). (H and E) X250.

Figure 2b: Magnified section of the figure (2a) showing undifferentiated connective tissues (arrows) and abnormal somniferous lobules with a large number of trapped spermatozoa (spz). (H and E) X 400.

In the cross-section of testes of *O. niloticus* control male reared at 36 ppt of salinity percentage of normal lobules was about 20% of the total lobules. In the cross-section of deformed *O. niloticus* testes, all spermatogenesis stages were detected with hypertrophied characters. The collagenous connective tissue fibers were detected in some places of the testes. These fibers increased in the interlobular connective

tissue as well as pigmentation. Elongated nucleoli of connective tissue cells are usually found in association with the fibers, as indicated in (Figure 3a). Other places of the testes were characterized by the presence of vacuoles spaces free from spermatogenesis (no detection of secondary spermatocytes), and moderate numbers of spermatozoa were detected as indicated in (Figure 3b).



3a

3b

Figure 3a: Photomicrograph of cross-section (CS) in the testes of the control group of *O. niloticus* reared at 36 ppt of salinity, showing collagenous connective tissue fibers (arrows), spermatocyte (Spc), spermatid (Spd) and spermatozoa (Spz). (H and E) X 250.

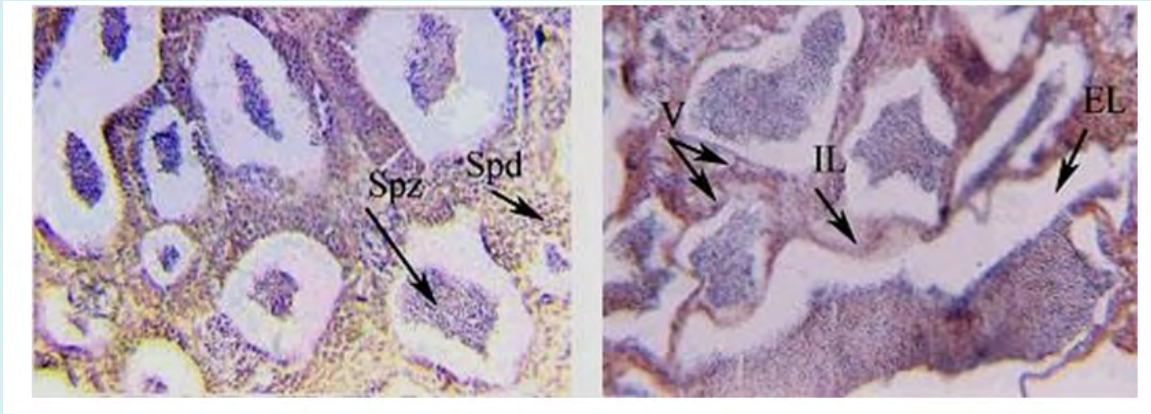
Figure 3b: Magnification of Fig. (3a) showing spaces free from spermatogenesis (arrows) and absence of interlobular connective tissue. (H and E) X400.

The percentage of normal lobules of genetically modified *O. niloticus* treated with sea bream DNA reared in freshwater was about 28% from total cells. The deformed testes in cross-section were characterized by some deflection of

interlobular tissue from its original position. In some places, the interlobular tissues were absent, and in other places, the interstitial cells were active spermatogonia observed. Also, the testes were characterized with different spermatogenic

activities, and the spermatozoa were discharged gradually from somniferous lobules as indicated in (Figure 4a). There

are many elongated lobules without detectable margins (Figure 4b).



4a

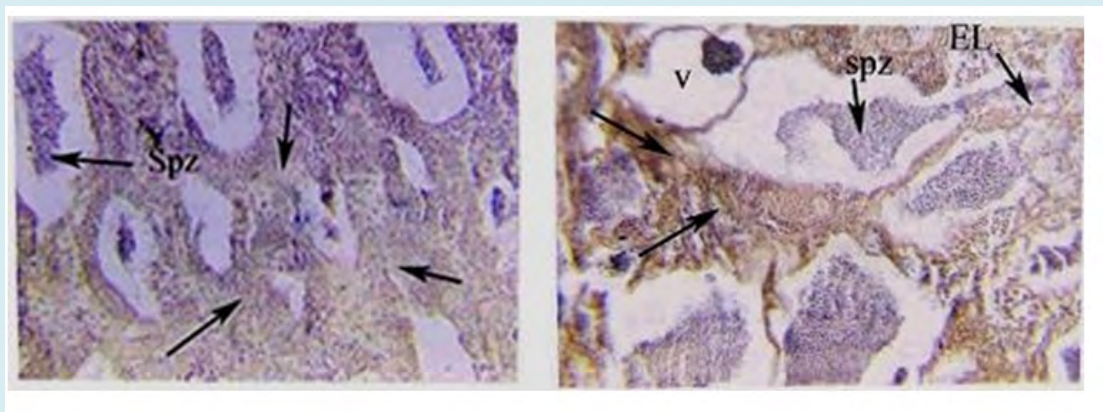
4b

Figure 4a: Photomicrograph of cross-section (CS) in the testes of genetically modified *O. niloticus* treated with sea bream DNA and reared at freshwater, showing absence of interlobular tissue, a moderate number of spermatozoa (Spz) and spermatid (Spd). (H and E) X250.

Figure 4b: Magnification of Fig. (4a), showing, deformed of Interlobular connective tissue (IL) and in some places absence of different stages of spermatogenesis, vacuoles (V), fibres and elongated lobules (EL). (H and E) X400.

In cross-section in testes of genetically modified *O. niloticus* treated with sea bream DNA reared at 18 ppt of salinity, the percentage of normal lobules was about 24% of the total lobules. The testes were characterized mainly by the presence of atretic lobules with abnormal and hypertrophy interlobular connective tissue. These lobules associated with more or less moderate or few numbers of spermatozoa, as shown in (Figure 5a). Other abnormal testes

were characterized by increased thickness of interlobular connective tissue to reach about 78 μm and elongation of seminiferous (Figure 5b). A random distribution of lobules with Undifferentiated boundaries with the elongated nucleus of collagenous connective tissue fibrous. The only spermatozoon was detected in the thin seminiferous lobules, as indicated in (Figure 5b).



5a

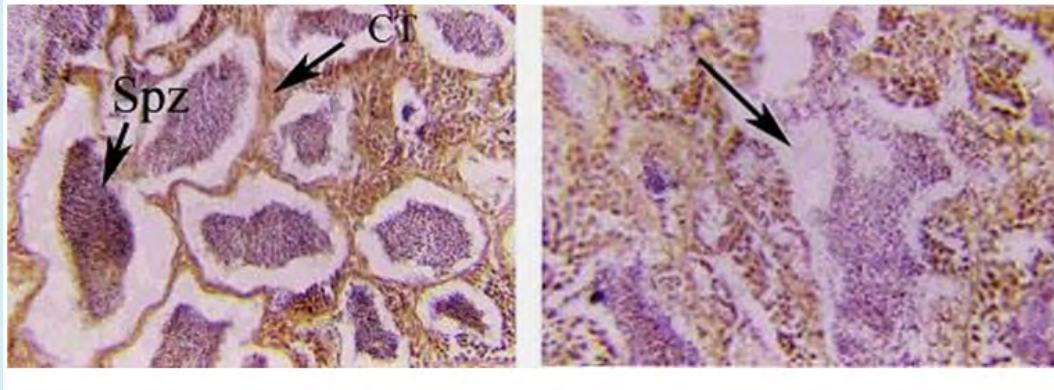
5b

Figure 5a: Photomicrograph of cross-section (CS) in the testes of genetically modified *O. niloticus* treated with sea bream DNA and reared at 18 ppt of salinity, showing absence of interlobular tissue, a moderate number of spermatozoa (Spz) and spermatid (Spd). (H and E) X250.

Figure 5b: Magnification of Fig. (5a), showing, deformed of Interlobular connective tissue (IL) and in some places absence of different stages of spermatogenesis, vacuoles (V), fibers and elongated lobules (EL). (H and E) X400.

The percentage of normal lobules genetically modified *O. niloticus*, treated with sea bream DNA reared at 36 ppt of salinity was about 10% of the total cells. The deformed testes were characterized by increased thickness of interlobular connective tissue hypertrophied in some places (**Figure**

6a). In the section of testes, there were vacuoles and spaces free from spermatogenesis. The trapped spermatozoa were intermingled with undifferentiated tissue as indicated in (**Figure 6b**).



6a

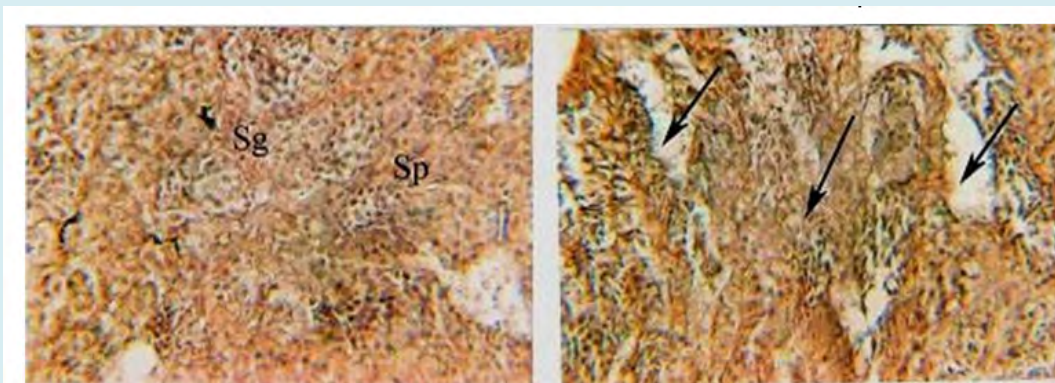
6b

Figure 6a: Photomicrograph of cross-section (CS) in the testes of genetically modified *O. niloticus* treated with sea bream DNA and reared at 36 ppt of salinity, showing increase thickness of interlobular connective tissue (arrow) in the section and largely trapped spermatozoa (Spz). (H and E) X 250.

Figure 6b: Magnified section of figure (6a) showing elongated seminiferous lobules with a large amount of trapped sperms (arrows) (H and E) X 400.

In cross-section of testes of genetically modified *O. niloticus* treated with *Artemia* DNA reared at freshwater, the percentage of normal lobules was about 40% of the total lobules. Also, spermatogonia and primary spermatocytes were only detected. The deformed testes were characterized by the absence of interlobular connective tissue and other different stages of spermatogenesis. In addition, there were

vacuoles and spaces free from spermatogenesis as indicated in (Figures 7 a,b). The results showed that undifferentiated interlobular connective tissue marked atrophic changes were observed. The testicular capsule becomes thickened and fibrosed, which is considered a sign of infertility and shortage in the secretion of gonadal hormones.



7a

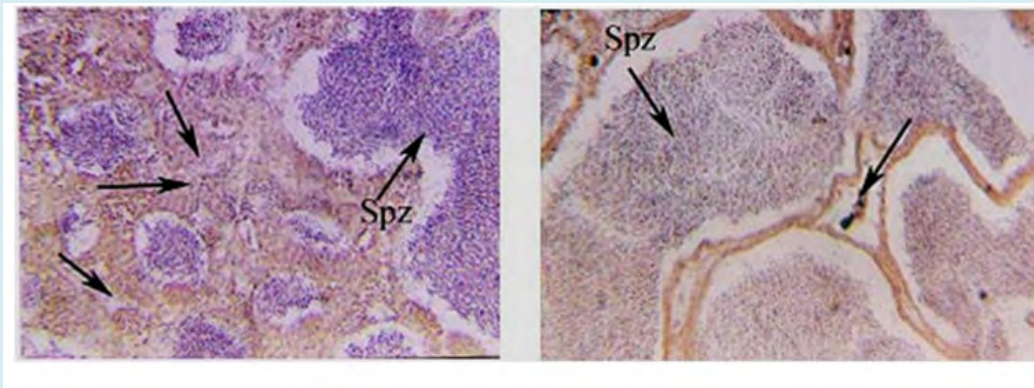
7b

Figure 7a: Photomicrograph of cross-section (CS) in the testes of genetically modified *O. niloticus* treated with *Artemia* DNA and reared at freshwater, showing: atretic lobules with only spermatogonia (Sg) and primary spermatocyte (sp) detected. (H and E) X 250.

Figure 7b: Photomicrograph of cross-section (CS) in the testes of genetically modified *O. niloticus* treated with *Artemia* DNA and reared at freshwater, showing spaces free from cells (arrows) and undifferentiated tissues (arrows). (H and E) X 400.

The percentage of normal lobules of genetically modified *O. niloticus* treated with ArtemiaDNA reared at 18 ppt of salinity was about 38% of the total cells from the light microscopic observation. The hypertrophy in some parts of interlobular connective tissue with an elongated nucleus and abnormal distribution and other treated testes were characterized with large elongated lobules with sharp boundaries (Figure 8a). Testes were characterized by a large

number of spermatozoa cells and undifferentiated tissue in the cross-section. Seminiferous tubules were noticed with the discharged amount of sperms as indicated in (Figure 8b). Testes of genetically modified *O. niloticus* treated with Artemia DNA reared at 18 ppt showed a large number of trapped spermatozoa and disintegrated interlobular connective tissue with atretic spaces between the lobules as indicated in (Figure 8b).

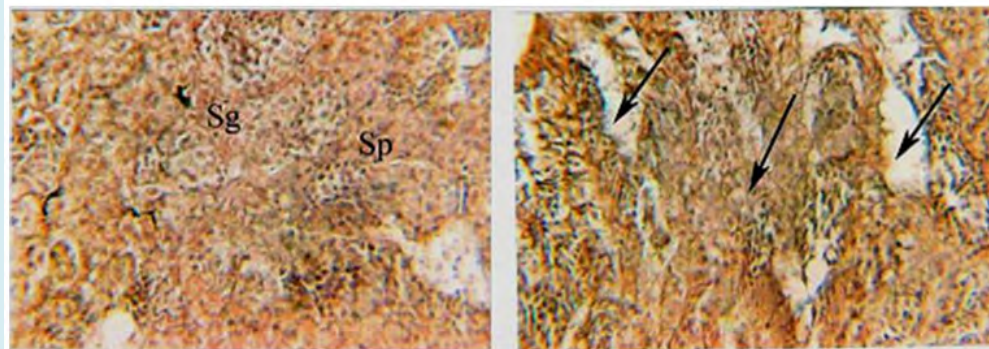


8a

8b

Figure 8a: Photomicrograph of cross-section (CS) in the testes of genetically modified *O. niloticus* treated with Artemia DNA and reared at 18 ppt of salinity, showing only a large amount of trapped ripe sperms in the seminiferous lobules (Spz) and hypertrophied interstitial connective tissues were detected (arrows) X 250.

Figure 8b: Magnified section of fig (8a) showing elongated seminiferous lobules with sharp boundaries, trapped spermatozoa (Spz), atretic space between lobules. (H and E) X 400.



9a

9b

Figure 9a: Photomicrograph of cross-section (CS) in the testes of genetically modified *O. niloticus* treated with Artemia DNA and reared in freshwater, showing: atretic lobules with only spermatogonia (Sg) and primary spermatocyte (sp) detected. (H and E) X 250.

Figure 9b: Photomicrograph of cross-section (CS) in the testes of genetically modified *O. niloticus* treated with Artemia DNA and reared in freshwater, showing spaces free from cells (arrows) and undifferentiated tissues (arrows). (H and E) X 400.

The percentage of normal lobules of genetically modified *O. niloticus* treated with ArtemiaDNA reared at 36 ppt was about 18% of the total cells. A large number of hypertrophied were observed in the interlobular connective tissue (Figure 9a). Spermatozoa were moderately detected with complete

degeneration of interlobular connective tissue (Figure 9b). Other parts of the testes were characterized by the absence of interlobular connective tissue, with thin tubular atretic seminiferous tubules (Figure 9b). Atretic tissue and vacuoles were detected in many spaces between spermatogenic cells

(Figure 9a,b). Also, the results showed that hypertrophied or deformed interlobular connective tissue was considered a good sign of infertility and a shortage of gonadal hormones.

Genotype Analysis (ISSR Fingerprinting Analysis)

All DNA samples from genetically modified *O. niloticus* treated with different types of DNA and their control were examined using an ISSR marker. Five primers were used to determine DNA fingerprinting in genetically modified *O. niloticus* treated with Artemia- DNA and control fish. The results showed that no amplification was detected in the

control reactions (without DNA source). All amplification products were reproducible when reactions were repeated using the same reaction conditions (Figure 10). The results also showed that the number of amplified bands detected varied, depending on the primers and DNA treatment. The highly genetic polymorphic percentage ranged from (11.20 to 34.20%) using different ISSR primers (Table 2 and Figure 10). It may be due to the differences in DNA molecule among normal and modified fish due to direct injection of foreign DNA isolated from Artemia. Moreover, some fragments of foreign DNA may be randomly integrated into *O. niloticus* genomes.

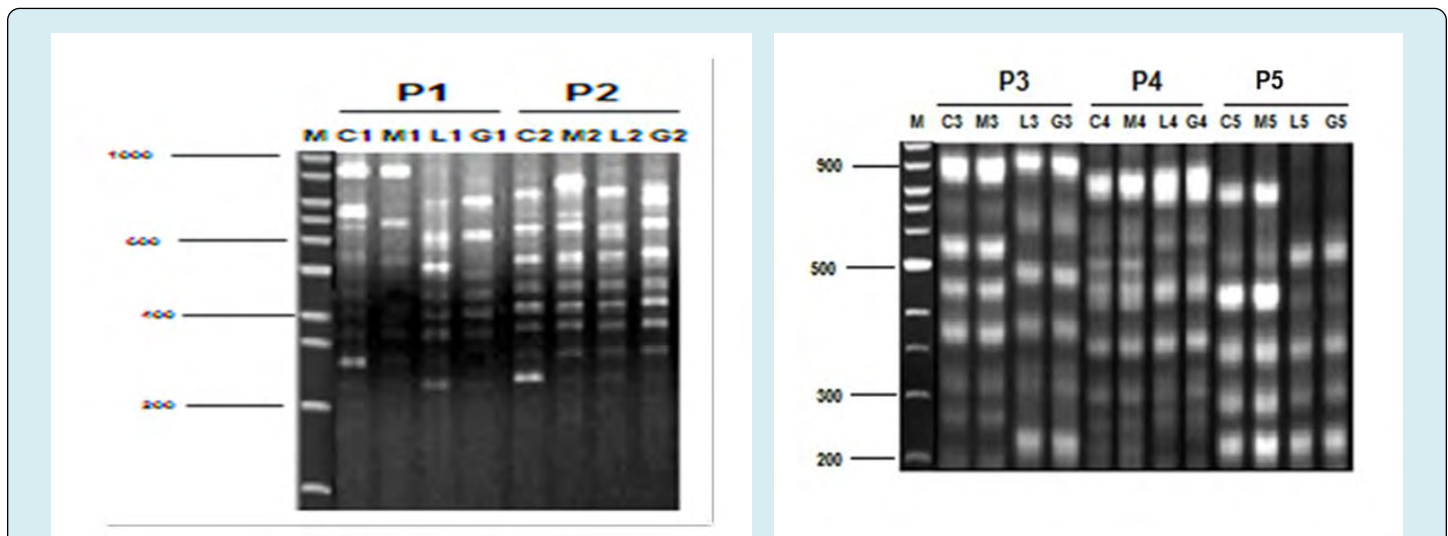


Figure 10: ISSR products generated from genetically modified *O. niloticus* treated with Artemia- DNA and their control fish, using five primers. Lane M: DNA marker, lanes C, M, L and G are control, muscles, liver and gonad tissues, respectively.

	Sample type											
	Control			Muscles			Liver			Gonads		
Primers	Total bands	Polymorphic bands	Polymorphism %	Total bands	Polymorphic bands	Polymorphism %	Total bands	Polymorphic bands	Polymorphism %	Total bands	Polymorphic bands	Polymorphism
1	20	9	45	22	7	31	23	15	65	22	9	40
2	24	4	16	26	2	7	30	6	20	29	12	41
3	26	2	7	27	2	7	26	3	23	26	6	23
4	26	9	34	26	3	11	22	5	22	26	10	38
5	19	1	5	18	0	0	17	2	11	17	5	29
Average % Polymorphm		21.4			11.20			28.20			34.20	

Table 2: Total amplified, Polymorphic, Monomorphic and Polymorphism % among Control, Muscles, Liver and Gonads of genetically modified *O. niloticus* reared in 36 ppt.

Discussion

Anthracyclines, Assem SS & SY EZ [18,19] reported that the effects of direct intramuscular injection of foreign DNA into *O. niloticus* or *T. zillii* could be limited to germ cells of fish. Testes of males injected with various amounts of foreign DNA showed a large number of abnormalities. They add that further studies about the establishment of these effects on the following generations are needed. Moreover, Assem SS, et al. [20] stated that the effects of the transfer of foreign DNA into *T. zillii* gonads on the histological changes of testes of the first and second generations derived from fast-growing genetically modified redbelly tilapia were studied. The testes of F1 were hypertrophy in the interlobular connective tissue with abnormal distribution, while the second generation of F2, a large number of vacuoles with much atretic tissue, was observed. The abnormal tests of F2 showed a few spermatozoa and deformed interlobular connective tissue.

The present work results are consistent with these findings, since the abnormal shape of interlobular connective tissue was considered evidence of infertility and shortage in steroid hormone secretion as indicated by SY EZ [21]. Moreover, the damage of interlobular tissue was considered a good sign of infertility, and the fish cannot produce steroid needed for gametogenesis. Also, the abnormal of the interlobular connective tissue shows a shortage in function of these collagenous tissues [22]. El-Zaeem and Assem, et al. [18] reported that these collagenous connective tissue cells with elongated nuclei were usually associated with fibers. The results of the present work showed that undifferentiated interlobular connective tissue marked atrophic changes were observed. The testicular capsule becomes thickened, and fibrosis was considered a sign of infertility and shortage in the secretion of gonadal hormones. Cochran, et al. [23] postulated that the primary function of interlobular connective tissue is to produce steroid needed for gametogenesis and expression of secondary sexual characters. Fahmy, et al. [24] reported that any deformation in the interlobular connective tissue was considered as indication of disturbance and shortage in secretion of gonadal hormones in *Chrysichthys rueppelli*. Also, Hines, et al. [25] reported that abnormal shape of the interlobular connective tissue showed a shortage in function of these tissues and all the interlobular cells; these interstitial cells are involved in steroid synthesis carried out in steps. Also, the results showed that hypertrophied or deformed interlobular connective tissue was considered a good sign of infertility and shortage of gonadal hormones, as indicated by Cochran, et al. [23] and Hines, et al. [25], who postulated that the primary function of interlobular cells is to produce steroid needed for gametogenesis and expression of secondary sex characteristics.

The present work results also showed that male fertility of *O. niloticus* decreased as a result of introducing foreign DNA into the gonads of their parents. This decrease depends on the type of DNA described by [5], since the present work showed that *O. niloticus* received Artemia DNA and reared at freshwater displayed normal lobules of 40 %. In comparison, *O. niloticus* received DNA sea bream showed only 28 % of normal lobules. These results were consistent with the finding reported by Penman, et al. [26] that there was a trend towards increased mortality of rainbow trout eggs with increasing DNA concentration. Abdul Razak, et al. [27] reported that gonad somatic index (GSI) of transgenic Nile tilapia males had a lower value (0.0326) compared to non-transgenic siblings (0.0736). The present work results also showed that the fertility of males decreased with increasing salinity levels up to 32 ppt. Although all fish that received foreign DNA displayed low fertility, Nile tilapia received Artemia DNA showed more salinity tolerance and highest fertility than Nile tilapia received sea bream DNA. In this connection, El-Zaeem, et al. [5,28] stated that increasing salinity levels led to dropped Gonadosomatic index and nonsuccess of reproduction. Also, Fineman-Kalio, et al. [29] indicated that spawning of *O. niloticus* was inhibited by high salinity, and the gonad development and spawning occurred at salinities of 17-19 ppt. With increasing water salinity from 25 to 30 ppt, the onset of reproduction was delayed while reproduction stopped completely at salinity above 30 ppt. The specific characterization of the RAPD method (random, uncharacterized multiple genome loci; dominant nature of markers; and the possibility of migration of non-homologous bands) result in limitations based on RAPD analysis alone. Despite these limitations, the RAPD analysis can be used effectively to assess genetic variation among fish species [30]. The main advantages of RAPD and ISSR markers are the possibility of working with anonymous DNA, relatively low expense, fast and simple to produce [31].

The results obtained by El-Zaeem, et al. [28] suggested that hypersaline genetically modified *O. niloticus* with a higher growth rate which can be produced by the transfer of a foreign DNA isolated from sea bream and Artemia as a feasible and fast methodology compared to interspecific hybridization, which is one classical breeding methods [5]. According to these results, genetically modified *O. niloticus* treated with sea bream and Artemia-DNA surpassed most of the productive performance traits under different salinity levels up to 3 ppt. The present work results are consistent with these findings since the present work showed that *O. niloticus* received Artemia DNA and reared at freshwater displayed normal lobules of 40 %. In comparison, *O. niloticus* received DNA sea bream showed only 28 % of normal lobules. El-Zaeem, et al. [32] recommends using foreign DNA transformation to induce sterility in Nile tilapia males and

thus solve the problem of overpopulation in commercial pond cultures. The only limitation of this methodology may be the time required to handle and inject the broodstock. The results of the present work are consistent with these findings.

Finally, the results showed that males' fertility decreased with increasing salinity levels up to 36 ppt. This matched with Barman HK, et al. [33] that reported the high salinity affected the hematological, expression of some genes and histological features of Nile tilapia.

Conclusion

Our result revealed that the fertility of males reduced with rising salinity levels up to 36 ppt. in addition; *O. niloticus* received Artemia DNA displayed normal lobules of 40 %. In contrast, *O. niloticus* received DNA sea bream showed only 28 % of normal lobules. Corresponding to these results, genetically modified *O. niloticus* received sea bream, and Artemia-DNA outstripped most of the productive performance traits under different levels of salinity up to 3 ppt.

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