World Journal of Fish and Marine Sciences 4 (6): 637-644, 2012
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2012.04.06.658

Effect of Dietary 17$-Estradiol on Serum Sex Hormones' Levels and Gamete Quality in Goldfish (*Carassius auratus*)

Reza Tarkhani, Mohammad Reza Imanpoor and Vahid Taghizadeh

Department of Fisheries, Faculty of Fisheries and Environment,
Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: Effect of dietary 17 $-estradiol (17E) on serum sex hormones and gamete quality was investigated in goldfish (*Carassius auratus*). Fish were fed diets containing 0, 10, 25 and 50 mg 17E kg dietG1 over a 193-day period. At the end of the trial, gamete quality as well as serum sex hormone levels were determined in both male and female fish. Results showed significant effect of 17E on both male and female gamete quality including increase in oocyte diameter, GSI, functional fecundity as well as decrease in oocyte surface: volume, spermatocrite, sperm density, motile sperm percentage and sperm motility duration. On the other hand fertilization and hatching rate as well as zygote diameter were higher in 17E-treated fish compared to control. Serum levels of estradiol and 17-hydroxyprogestrone were high in 17E-treated fish compared to the control group. However, the lower serum testosterone levels were found in 10 mg 17E kg dietG1 group, followed by the control group. In conclusion, dietary 17E increases oocyte diameter, oocyte surface: volume ration, GSI, functional fecundity in female and decreases semen quality in male goldfish. Likewise, egg diameter, fertilization and hatching rates improve as a result of 17E treatment. However it may cause some hormonal disturbance as well.

Key words: 17$-Estradiol %Carassius auratus % Gamete Quality % Sex Hormone

INTRODUCTION

Availability of the both sex in sufficient number is necessary for successful artificial propagation activity. Deficiency in either male or female individuals leads to fail to reach the predicted eggs as well as larvae, resulting in economic loss. Due to this, many efforts have been conducted to facilitate and ensure the availability of the each sex. Fish sperm cryopreservation is a method for ensuring male gamete availability which has been developed in many species [1-5] including goldfish [6]. On the other hand, dietary sex steroids have been used to achieve mono-sex population in some fish species [7-12].

Dietary 17$-estradiol (17E) has been found to feminize the fish population in some fish species [9-12]. This method can ensure the availability of the female individuals which allows the culturists to program their egg production in a better way. However, it has been shown that 17E treatment affect gamete quality, as well [13, 14]. Goldfish, *Carassius auratus*, is an ornamental fish and the most common one in Iran. Its importance for Iranians refers to its necessity for the ancient Nowruz Holyday. Due to this high market demand for this species, breeding and rearing of this species is performed by many people in potential regions of Iran and brings reasonable profit for employers who many of them are completely satisfied with this job [15]. Goldfish is the most extensively studied species with respect to basic reproductive physiology and endocrine regulated behavior [16]. Our previous trial showed that long-term dietary 17E treatment could induce a successful mono-sex population in goldfish. However, gonad quality was not determined under such treatment. Thus, the present work aimed to determine the effect of dietary 17E treatment on gamete quality and serum sex hormones' levels in goldfish.

Corresponding Author: Reza Tarkhani, Faculty of Fisheries and Environment, Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
Tel: +989371140226, Fax: +981712245886.

MATERIALS AND METHODS

Diets: The control diet composition is presented in Table 1. To prepare the control diet, the ingredients were weighed and mixed carefully and moisturized by appropriate water amount to facilitate pellet forming. The obtained dough then passed through a metal mesh (1 mm in diameter) to form threads. Threads were air-dried and re-grounded into appropriate size and passed through a 0.5 mm mesh. To prepare the 17E-supplemented diets, 17E was purchased (estradiol valerate, Sigma, St. Louis, MO) and 10, 25 and 50 mg 17E were dissolved in 400 ml ethanol (96 %) and added to 1kg diet ingredients mixture (control diet) [11]. The mixtures were remained in room temperature for ethanol evaporation. Thereafter, the mixtures were moisturized with appropriate water amount and processed similar to the control diet to obtain the 0.5 mm particles. All diets were frozen (-18°C) until use.

Fish: The study was conducted using goldfish larvae. To obtain demanded larvae, goldfish brood stocks (30 g) were injected by Ovaprim for artificial propagation. Oocytes and milt were stripped and mixed together using a light feather. After adhesion removal (2 ppt NaCl solution), the obtained eggs were transferred into glass aquarium supplied with gentle aeration. After 48 h the eggs were hatched (at 23°C) with the hatching rate of 95 %. Exogenous feeding (dry milk, green water and artemia naupli) was started at 72 h post hatch. The larvae were fed on by the mentioned foods over a 4-month period. Survival and average weight were 20 % and 0.1 g at the end of this period.

Experimental Design: A total of 180 larvae (0.12±0.002g) were randomly distributed into 4 treatments (control, 10, 25 and 50 mg 17E kg diet^{-1}), each contains 3 fiberglass tanks (300 L water) as replications. Fish were coffered the control diet over 10 days for adaptation and thereafter each treatment received its corresponding diets over a 193-day period. Water exchange was 80 % every other day. Water dissolved oxygen, pH, temperature and total hardness were 6.5±0.7 mg L^{-1}, 7.2±0.3, 25±1.4°C and 200±15.2 mg L^{-1}. Photoperiod was natural (March-October, 2011).

At the end of the trial, 5 male and 5 female fish were sampled from each treatment to evaluate the gamete characteristics. In the male individuals, seminal pH, spermatocrit, sperm motility duration, motile sperm percentage, sperm density and collectable-milt volume were recorded, whereas, in the female ones, oocyte diameter, oocyte's surface: volume ratio, functional fecundity and gonadosomatic index (GSI) were checked.

Table 1: Control diet ingredients

Ingredient	g/kg diet^{-1}
Fish meal	205.5
Soybean meal	385
Wheat meal	101
Corn meal	61
Rice bran	187.5
Fish oil	5
Vitamin mix[#]	20
Mineral mix[$]	20
Lysine	7.5
Methionine	7.5
Proximate composition	
Protein (%)	39
Lipid (%)	10.8
Moisture (%)	6
Gross energy kcal kg^{-1}	4000

[#] Vitamin mix was formulated to provide the following amounts for diet (mg kg^{-1} of diet): B_1, 0.7; B_2, 10; B_3, 40; B_5, 40, B_6, 9; B_9, 3; B_{12}, 0.01; biotin, 1.5; vitamin K, 4.5; vitamin A, 6000 IU; vitamin E, 150 IU. All vitamins were purchased from companies Adisseo (Antony, France) and BASF (Ludwigshafen, Germany).

[$] Mineral mix was formulated to provide the following amounts for diet (mg kg^{-1} of diet): Mg, 500; Fe, 150; Zn, 20; Mn, 13; Cu, 4.8; Co, 0.1. All minerals were purchased from Company of Divan shimi (Tehran, Iran).

Likewise, 5 females were randomly captured from each treatment and anesthetized (clove solution 3000 ppm) and blood-sampled by caudal severance. Blood samples were collected in non-heparinized tubes and centrifuged (5000 rpm, 5 min) to attain serum. Serum samples were analyzed for 17E, 17-Hydroxyprogesterone (17 OH) and testosterone determination.

Thereafter, five females (6-8 g) from each treatment were sampled and distributed into the five separate aquaria. Likewise, 5 males (6 g) fish were captured from the control group and stocked in another aquaria. All aquaria containing male and female fish were subjected to a temperature treatment as follow: initial temperature was 20°C which decreased to 10°C over a 10-day period (1°C per day). Fish were kept at 10°C over another 10-day period and thereafter temperature was increased to 20°C over a-10-day period (1°C per day). After temperature treatment, the female fish were intramuscular-injected by Ovaprim (0.2 ml). Eight hours afterwards, females were injected (0.2 ml) as above. Simultaneous with the second injection in the females, the males were once injected (0.2 ml). Seventeen hours after the second injections, gametes were attained by

hand-stripping. Eggs obtained from all treatments were fertilized by the mixed milt collected from the males (5 fish). Oocytes and milt were stripped and mixed together using a light feather. After adhesion removal (2 ppt NaCl solution), the obtained eggs were transferred into glass aquarium supplied with gentle aeration. After 48 h the eggs were hatched (at 23°C). Fertilization rates as well as hatching rates were determined in each treatment.

Gonad Characteristic Determination

Female: Oocyte diameters were determined using a scaled loop with 40 sub-replications for each treatment replication (tank). The average of the sub-replications was considered as one replicate of each treatment. Oocytes surface and volume were calculated according to the diameter. To estimate the functional fecundity, the weights of the total obtained oocytes for each fish (five fish per treatment) were recorded. Three fractions (~ 0.2 g) were detached from each gonad and the numbers of the oocytes were determined under a loop. Accordingly, functional fecundity was determined as follow: FF = MONF × GW / MFW

Where,

FF = functional fecundity,
MONF = Mean oocyte number of the 3 fraction of gonad,
GW = Gonad weight,
MFW = Mean weight of the 3 fraction of gonad.
GSI = was calculated as follow:
GSI = Gonad mass / body mass

Male: Seminal pH was determined using a semi-microelectrode (SM102 pH Meter). Spermatocrit was measured for each milt sample by filling two capillary tubes with milt and centrifuging them for 10 min in a microhematocrit (12000 rpm) and expressed as percentage. Collectable-milt volume was determined gravimetrically. Sperm density was calculated by multiplying the collectable-milt volume to spermatocrit value.

To assess the sperm motility characteristics, 5 µl of milt was taken in a clean pipette, added to 1•5 ml of one of the activating solutions at 5°C and stirred vigorously. A drop of the diluted and activated sperm sample was immediately added to the chamber of a Neubauer hemacytometer and sperm activity was recorded by digital camera (Canon IXUS 70, www.canon.com). The sperm characteristics were determined using these records as follow:

- Sperm motility duration: to determine the maximum duration of motility of spermatozoa, the elapsed time was measured from the moment sperm were activated until none of the sperm in the viewing grid were moving forward.
- Motile sperm percentage: to determine the motile sperm, recorded videos were checked and 30 randomly selected sperm were checked for motility, after activation. Motile sperm percentage was obtained by dividing the number of motile sperm to the whole sperm number multiplied by 100.

Fertilization and Hatching Rates: After mixing of the oocytes with the sperm of control fish, 10 batches of each treatment's eggs (100 eggs) were transferred the 10 Petri dishes and the number of eyeing eggs was recorded after 24h. The fertilization rates were calculated by dividing of the number of eyeing eggs to 100 multiplied by 100, for each Petri dish. The values were presented as the treatments' mean±SD.

To determine hatching rates, aforementioned above procedure was conducted and the number of hatched eggs were recorded. Hatching rates were calculated by dividing of the number of hatched eggs to 100 multiplied by 100, for each Petri dish. The values were presented as the treatments' mean ±SD.

Serum Hormone Levels: Serum 17E and testosterone (Neogen Co. Lexington, KY, USA) as well as 17OH (IBL International, Hamburg, Germany) levels were measured by ELISA method.

Statistical Analyses: Data were subjected to one way ANOVA and Duncan's test to determine significant difference at $P<0.05$. Data are presented as mean±SD.

RESULTS

No mortality was observed during the trial. Since all fish in 25 and 50 mg 17E kg diet^{-1} groups were found to be female, male gamete quality were compared only between the control and 10 mg 17E kg diet^{-1} group.

Results showed significant effect of dietary 17E on female gamete quality ($P<0.05$). Oocyte diameter was higher in 25 and 50 mg 17E kg diet^{-1} groups compared to the control and 10 mg 17E kg diet^{-1} group (Fig. 1).

Oocyte surface: volume ratio in 50 mg 17E kg diet^{-1} group was significantly lower than control and 10 mg 17E kg diet^{-1} groups, however, there was no significant

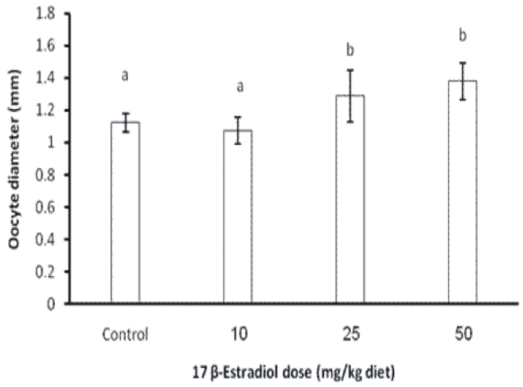

Fig. 1: Effect of dietary17 $-estradiol on oocyte diameter in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

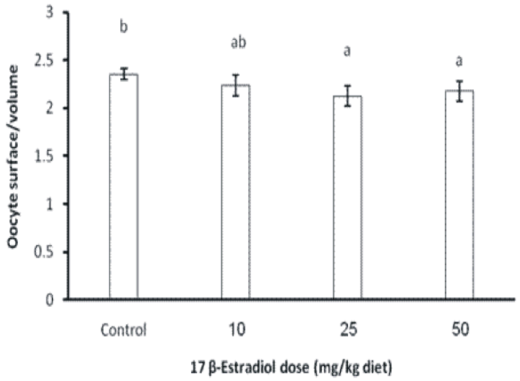

Fig. 2: Effect of dietary 17 $-estradiol on oocyte surface: volume in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

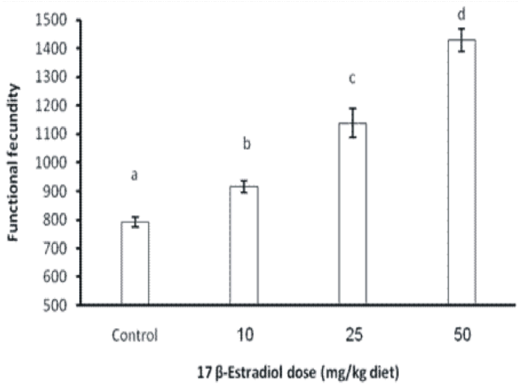

Fig. 3: Effect of dietary17 $-estradiol on functional fecundity in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

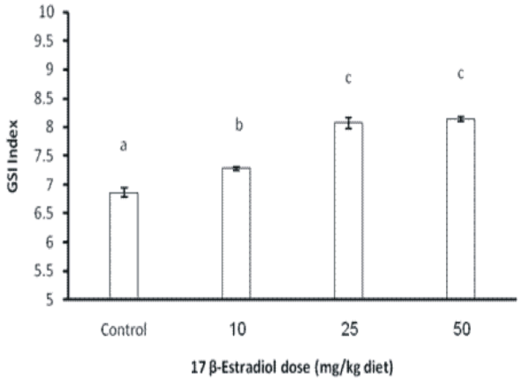

Fig. 4: Effect of dietary17 $-estradiol on GSI in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

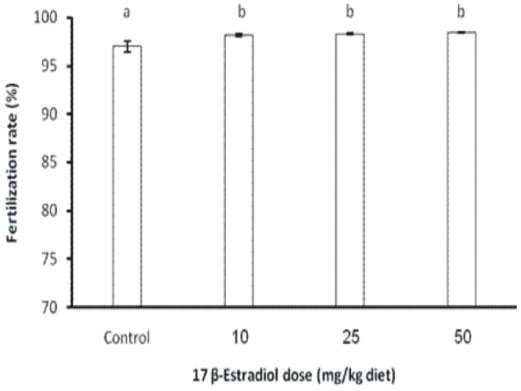

Fig. 5: Effect of dietary17 $-estradiol on fertilization rate in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

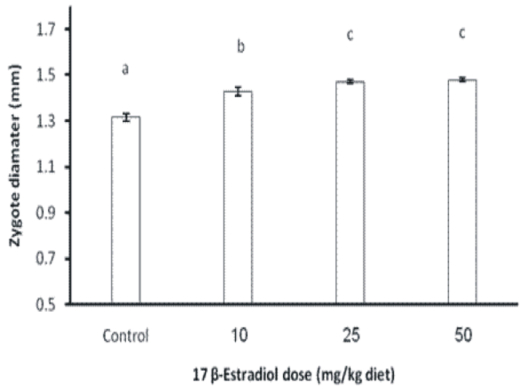

Fig. 6: Effect of dietary17 $-estradiol on zygote diameter in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

difference in between 25 mg 17E kg dietG^{1} group and the other groups (Fig. 2). Functional fecundity significantly increased whereas dietary 17E increased (Fig. 3).

GSI was not significantly different between 25 and 50 mg 17E kg dietG^{1} groups being significantly higher than control and 10 mg 17E kg dietG^{1} group (Fig. 4). 10 mg 17E

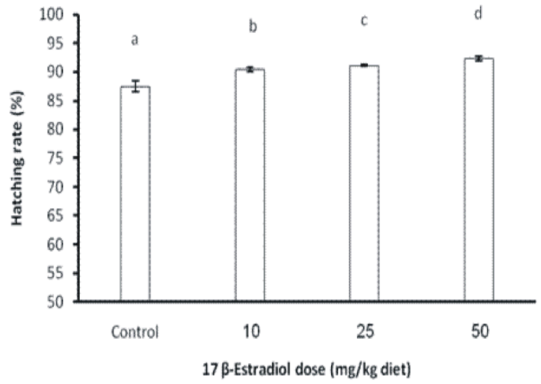

Fig. 7: Effect of dietary17 $-estradiol on hatching rate in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

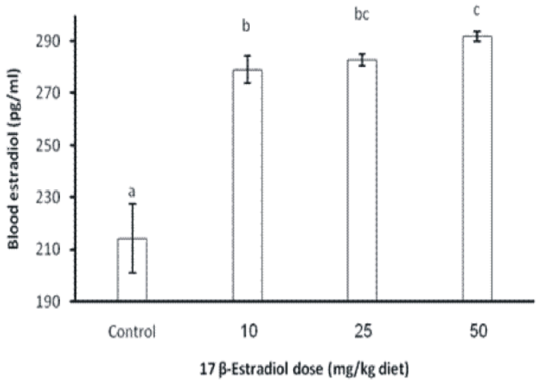

Fig. 8: Effect of dietary17 $-estradiol on blood estradiol levels in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

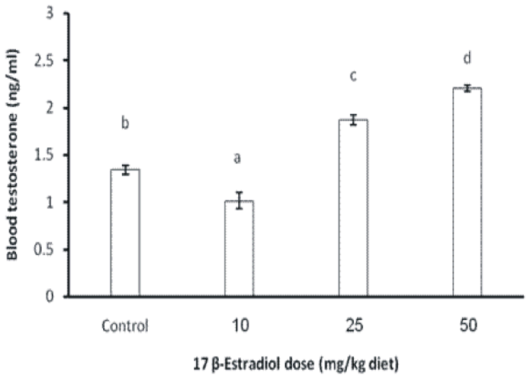

Fig. 9: Effect of dietary17 $-estradiol on blood testosterone levels in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

kg dietG^{1} group showed significantly higher GSI compared to control group (Fig. 4). Oocyte diameter increases along with gonad development.

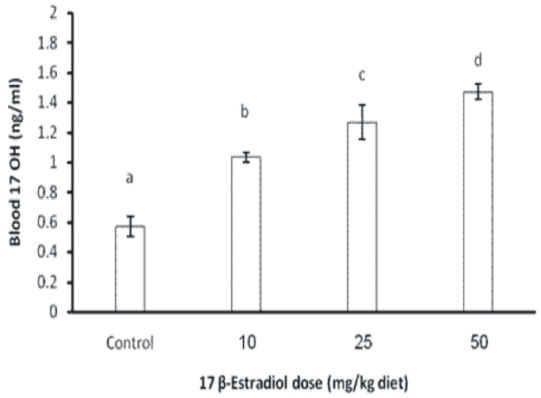

Fig. 10: Effect of dietary17 $-estradiol on blood 17 hydroxyprogestrone levels in goldfish. Different letters above the bars show significant difference ($P < 0.05$)

Table 2: Effect of dietary17 $-estradiol on male gamete quality in goldfish

	Control	10 mg 17 $-estradiol
Seminal pH	8.28 ± 0.06 a	8.32 ± 0.03 a
Spermatocrit (%)	35.34 ± 0.37 a	32.44 ± 0.21 b
Motile sperm percentage (%)	88.62 ± 2.6 a	85.36 ± 1.2 b
Sperm density (× 10^{9} mlG^{1})	6.12 ± 0.07 a	5.78 ± 0.08 b
Motility duration (s)	104 ± 4.3 a	96 ± 1.87 b
Milt volume (ml)	0.48 ± 0.11 a	0.44 ± 0.10 a

There was no significant difference in seminal pH and milt volume between the control and 10 mg 17E kg dietG^{1} group (Table 2). However, spermatocrit, motile sperm percentage, sperm density and motility duration were significantly low in 10 mg 17E kg dietG^{1} group compared to the control (Table 2).

Zygote diameter was not significantly different between 25 and 50 mg 17E kg dietG^{1} groups being significantly higher than control and 10 mg 17E kg dietG^{1} group (Fig. 5). 10 mg 17E kg dietG^{1} group showed significantly higher zygote diameter compared to control group (Fig. 5).

Fertilization rate was not significantly different between the 17E groups, being significantly higher than the control group (Fig. 6). Hatching rate significantly increased whereas dietary 17E increased (Fig. 7).

Serum 17E levels were significantly high in the 17E groups compared to the control group (Fig. 8). 50 mg 17E kg dietG^{1} group showed higher 17E levels compared to 10 mg 17E kg.diet-1 group, however, there was no significant difference between 25 mg 17E kg dietG^{1} group and the other 17E groups (Fig. 8).

Serum testosterone levels significantly decreased in 10 mg 17E kg dietG^{1} group compared to the other groups (Fig. 9). Control group showed lower testosterone levels

compared to 25 mg 17E kg diet^{-1} group which was significantly lower than 50 mg 17E kg diet^{-1} groups (Fig. 9). Serum 17 OH levels significantly increased as dietary 17E increased (Fig. 10).

DISCUSSION

The present result showed the importance of exogenous 17E on gamete quality. Gamete quality in turn, determines fertilization, hatching and larvae quality.

Increase in oocyte diameter, GSI and fecundity as well as decrease in oocyte surface: volume ration in 17E groups are believed to be related to estradiol. It has been previously found that estradiol has an important effect of ovary development in fish, [17-19] found correlation between serum estradiol levels and oocyte size and GSI in *Leptocottus armatus*. Likewise, [20] reported that estradiol modulating vitellogenin, the precursor to yolk proteins, synthesis and secretion by liver. Yolk size is one of the factors affecting oocyte diameter. Thus it seems that the higher oocyte size in 17E-treated fish is attributed to higher vitellogenin synthesis by liver as well as higher yolk size in the oocytes.

17E is reported to play a central role in the hypothalamus-pituitary-gonadal neuroendocrine axis regulating fecundity and oocyte development in fish [17,18]. Although relatively little is known about the exact mechanisms [21, 22], estrogens have been regarded as active on oogonial proliferation [21]. Higher functional fecundity in the present study in 17E-treated fish seems to be due to increment in ovulation rate. Ovulation is affected by maturation-inducing hormone, 17" 20$-Dihydroxy-4-pregnen-3-one [18, 23]. This hormone is synthesis from 17 OH by the action of the enzyme 20$ hydroxysteroid dehydrogenase [18, 23, 34]. Since higher levels of 17 OH were observed in 17E-treated fish, it might that the rate of the 17" 20$-Dihydroxy-4-pregnen-3-one synthesis increases which in turn extols the number of the ovulated oocytes. Previous studies on salmonids [13] and zebra fish [24] showed increase in the number of ovulated fish following estradiol therapy. Likewise, [25] showed increase in the mean number of spawned eggs as a result of exposure to low concentrations of estradiol in fathead minnow *Pimephales promelas*.

The investigated semen quality showed decline in 17E-treated fish. It is suggested that estradiol administration to male fish leads to the regression of testicular tissue and to the development of secondary ovaries (sex reversal) [23]. Complete sex reversal was observed in the 25 and 50 mg 17E kg diet^{-1} groups as no male individuals was present in these groups. Decline in semen quality was reported in male rainbow trout *Oncorhynchus mykiss* exposed to 17$-ethinylestradiol [26]. [13] reported decrease in semen volume, sperm density and semen fertility in rainbow trout *O. mykiss* exposed to 17E over 50 days. Decrease in the secondary sex characteristics was observed in male fathead minnows *P. promelas* after 17E exposure, including reduced nuptial breeding tubercles number as well as diameter [27].

Fish were sampled approximately at the end of the vitellogenesis stage for hormone assay. According to Hyllner *et al*. [28] 17E is responsible for vitellogenine synthesis in the liver, thus the levels 17E is assumed to be elevated during the vitellogenesis stage. However, the higher levels of serum estradiol in 17E-treated fish seem to be due to exogenous estradiol intake, because the 17 OH levels were high in these groups and elevation in this hormone shows initiation of final oocyte maturation and ovulation [29]. Likewise, the higher levels of 17OH might suggest 17E-treated fish have more developed oocyte than control group, since this hormone levels increase after vitellogenesis stage termination. However, testosterone pattern is hard to interpreting between the treatments. Testosterone has been suggested to be elevated during vitellogenesis, but drop during final maturation [30, 31]. Such pattern might be due to endocrine disruption effect of exogenous estradiol. Endocrine disruption effect of estradiol has been reported in previous studies [25, 26, 31, 32].

In conclusion, dietary 17E increases oocyte diameter, oocyte surface: volume ration, GSI, functional fecundity in female and decreases semen quality in male goldfish. Likewise, egg diameter, fertilization and hatching rates improve as a result of 17E treatment. However, it may cause some hormonal disturbance, as well.

REFERENCES

1. Harvey, B., R. Norman Kelley and M.J. Ashwood-Smith, 1982. Cryopreservation of zebra fish spermatozoa using methanol. Canadian Journal of Zoology, 60: 1867-1870.
2. Magyary, I., B. Urbányi and L. Horváth, 1996. Cryopreservation of common carp (*Cyprinus carpio* L.) sperm II. Optimal conditions for fertilization. Journal of Applied Ichthyology, 12: 117-119.
3. Linhart, O., M. Rodina and J. Cosson, 2000. Cryopreservation of sperm in common carp Cyprinus carpio: Sperm motility and hatching success of embryos. Cryobiology, 41: 241-250.

4. Suquet, M., C. Dreanno, C. Fauvel, J. Cosson and R. Billard, 2000. Cryopreservation of sperm in marine fish. Aquaculture Research, 31: 231-243.
5. Huang, C., Q. Dong, R.B. Walter and T.R. Tiersch, 2004. Sperm cryopreservation of green swordtail *Xiphophorus helleri*, a fish with internal fertilization. Cryobiology, 48: 295-308.
6. Babiak, I., E. Brzuska and J. Perkowski, 2000. Fractional factorial design of screening experiments on cryopreservation of fish sperm. Aquaculture Research, 31: 273-282.
7. Hishida, T. and N. Kawamoto, 1970. Androgenic and male-inducing effects of 11-ketotestosterone on a teleost, the medaka (*Oryzias latipes*). Journal of Experimental Zoology, 173: 279-283.
8. Owusu-Frimpong, M. and B. Nijjhar, 1981. Induced sex reversal in *Tilapia nilotica* (Cichlidae) with methyl testosterone. Hydrobiologia, 78: 157-160.
9. Park, I., J. Kim, S.H. Cho and D.S. Kim, 2004. Sex differentiation and hormonal sex reversal in the bagrid catfish Pseudobagrus fulvidraco (Richardson). Aquaculture, 232: 183-193.
10. Flynn, S.R. and T.J. Benfey, 2007. Effects of dietary estradiol-17$ in juvenile shortnose sturgeon, *Acipenser brevirostrum*, Lesueur. Aquaculture, 270: 405-412.
11. Wang, H., Z. Gao, B. Beres, J. Ottobre, G. Wallat, L. Tiu, D. Rapp, P. O'Bryant and H. Yao, 2008. Effects of estradiol-17$ on survival, growth performance, sex reversal and gonadal structure of bluegill sunfish *Lepomis macrochirus*. Aquaculture, 285: 216-223.
12. Zhang, H. and D. Jin, 2011. Effects of different exogenous estradiol contents on steroid hormones, GSI, survival rate and sex reversal in the Asian Swamp Eel. Advanced Materials Research, 382: 481-485.
13. Lahnsteiner, F. B. Berger, M. Kletzl and T. Weismann, 2006. Effect of 17$-estradiol on gamete quality and maturation in two salmonid species. Aquatic Toxicology, 79: 124-131.
14. Schubert, S., A. Peter, R. Burki, R. Schönenberger, M.J.F. Suter, H. Segner and P. Burkhardt-Holm, 2008. Sensitivity of brown trout reproduction to long-term estrogenic exposure. Aquatic Toxicology, 90: 65-72.
15. Hoseini, S.M. and R. Tarkhani, 2012. Effect of short term treatment with potassium permanganate on stress markers and blood biochemistry in goldfish *Carassius auratus*. Aquaculture Research doi: 10.1111/j.1365-2109.2012.03091.x
16. Bjerselius, R., K. Lundstedt-Enkel, H. Olsen, I. Mayer and K. Dimberg, 2001. Male goldfish reproductive behavior and physiology are severely affected by exogenous exposure to 17 $-estradiol. Aquatic Toxicology, 53: 139-152.
17. Campbell, B., J. Dickey, B. Beckman, G. Young, A. Pierce, H. Fukada and P. Swansonm, 2006. Previtellogenic oocyte growth in salmon: relationships among body growth, plasma insulin-like growth factor 1, estradiol-17beta, follicle-stimulating hormone and expression of ovarian genes for insulin-like growth factors, steroidogenic acute regulatory protein and receptors for gonadotropins, growth hormone and somatolactin. Biology of Reproduction, 75: 34-44.
18. Nagahama, Y., 1994. Endocrine regulation of gametogenesis in fish. International Journal of Development Biology, 38: 217-229.
19. DeVlaming, V., R. Fitzgerald, G. Delahunty, J.J. Cech Jr, K. Selman and M. Barkley, 1984. Dynamics of oocyte development and related changes in serum estradiol-17$, yolk precursor and lipid levels in the teleostean fish, *Leptocottus armatus*. Comparative Biochemistry and Physiology Part A: Physiology, 77: 599-610.
20. Specker, J.L. and C.V. Sullivan, 1994. Vitellogenesis in fishes: status and perspectives. In: Perspectives in Comparative Endocrinology (ed. by K.G. Davey, R.E. Peter and S.S. Tobe), pp: 304-315. Ottawa: National Research Council of Canada.
21. Jalabert, B., J.F. Baroiller, B. Breton, A. Fostier, F. Le Gac, Y. Guiguen and G. Monod, 2000. Main neuro-endocrine, endocrine and paracrine regulations of fish reproduction and vulnerability to xenobiotics. Ecotoxicology, 9: 25-40.
22. Tyler, C.R. and J.P. Sumpter, 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries, 6: 287-318.
23. Devlin, R.H. and Y. Nagahama, 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological and environmental influences. Aquaculture, 208: 191-364.
24. Brion, F., C.R. Tyler, X. Palazzi, B. Laillet, J.M. Porcher, J. Garric and P. Flammarion, 2004. Impacts of 17$-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (*Danio rerio*). Aquatic Toxicology, 68: 193-217.

25. Jobling, S., D. Casey, T. Rodgers-Gray, J. Oehlmann, U. Schulte-Oehlmann, S. Pawlowski, T. A.P. Baunbeck, Turner and C.R. Tyler, 2003. Comparative responses of molluscs and fish to environmental estrogens and estrogenic effluent. Aquatic Toxicology, 65: 205-220.
26. Schultz, I.R., A. Skillman, J.M. Nicolas, D.G. Cyr and J.J. Nagler, 2003. Short-term exposure to 17$-ethynyl estradiol decreases the fertility of sexually maturing male rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry. 22: 1272-1280.
27. Miles-Richardson, S.R., V.J. Kramer, S.D. Fitzgerald, J.A. Render, B. Yamini, S.J. Barbee and J.P. Giesy, 1999. Effects of waterborne exposure of 17 $-estradiol on secondary sex characteristics and gonads of fathead minnows (*Pimephales promelas*). Aquatic Toxicology, 47: 129-145.
28. Hyllner, S.L., B. Norberg and C. Haux, 1994. Isolation, partial characterisation, induction and the occurrence in plasma of the major vitelline envelope proteins in the Atlantic halibut (*Hippoglossus hippoglossus*) during sexual maturation. Canadian Journal of Fisheries and Aquatic Sciences, 51: 1700-1707.
29. Peter, R.E. and K.L. Yu, 1997. Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. Reviews in Fish Biology and Fisheries, 7: 173-197.
30. Kime, D.E., 1993. 'Classical' and 'non-classical' reproductive steroids in fish. Reviews in Fish Biology and Fisheries, 3: 160-180.
31. Linard, B., I. Anglade, S. Bennani, G. Salbert, J.M. Navas, T. Bailhache, F. Pakdel, P. Jego, Y. Valotaire, C. Saligaut and O. Kah, 1995. Some insights into sex steroid feedback mechanisms in the trout. In: Reproductive Physiology of Fish. (ed. by F.W. Goetz and P. Thomas). pp. 49-51, Austin, TX: Fish Symposium, pp: 95.
32. Nagahama, Y., 1997. 17", 20$-Dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: Mechanisms of synthesis and action. Steroids, 62: 190-196.
33. Naji, T., P.Nejatkhahmanavi and M. Shirinabadi, 2009. The investigation of the effects of 17$-estradiol on gonadal differentiation in rainbow trout *Onchorhynchus mykiss*. Environmental Sciences and Technology, 10: 105-112. (in Persian)