Lat. Am. J. Aquat. Res., 45(5): 930-936, 2017
DOI: 10.3856/vol45-issue5-fulltext-8

Research Article

Levels of 17β-estradiol, vitellogenin, and prostaglandins during the reproductive cycle of *Oreochromis niloticus*

Juan Ortiz Tirado[1], Luis Valladares[2], Daysi Muñoz[1], Jairo Caza[1] Bangeppagari Manjunatha[1,3] & Rajesh R. Kundapur[4]
[1]Department of Life Sciences, Laboratory of Water Resources and Aquaculture
Universidad de las Fuerzas Armadas-ESPE, Sangolquí, Ecuador
[2]Laboratory of Hormones and Receptors, Institute of Nutrition and Food Technology (INTA)
University of Chile, Santiago, Chile
[3]Center for Biofluid and Biomimic Research, Department of Mechanical Engineering
Pohang University of Science and Technology (POSTECH), Pohang South Korea
[4]Department of Zoology, University of Pune, Pune, India
Corresponding author: Juan Ortiz Tirado (jcortiz@espe.edu.ec)

ABSTRACT. Circulating levels of estradiol, vitellogenin and prostaglandins could be references of oocytes quality in the aquaculture industry. This study evaluated the performance of 17β-estradiol (E2) and vitellogenin (Vtg) levels in plasma and prostaglandins in oocytes (PGE 2) during the reproductive period of *Oreochromis niloticus* in a commercial farm in Ecuador. Adults fish females were treated with estradiol-synthetic (ES) and tilapia pituitary extracts (TP) during 25 days. For quantification of E2 and PGE 2, an enzyme-linked immunosorbent assay (ELISA) was used, while for Vtg relative quantification a Western blot analysis was performed. Circulating E2 concentrations were different during the first week of follow-up ($P < 0.05$) resulting in different levels of Vtg per treatment ($P < 0.05$) affecting on gonadal growth. The ovaries had an asynchronous development, where the mature oocytes proportions were higher with ES and TP than control. Relative fecundity was different between treatments ($P < 0.05$), where TP generated 15.7 ± 5.8 oocytes per g of spawning female. Furthermore, concentrations of PGE 2 were different between treatments ($P < 0.05$). The information reported in this study will be useful to improve the reproduction methods and have some biological signals before ovulation in tilapia commercial culture.

Keywords: *Oreochromis niloticus*, tilapia, estradiol, vitellogenin, pituitary, prostaglandin E2.

INTRODUCTION

White shrimp (*Litopenaeus vannamei*) is the main species in the aquaculture in Ecuador, with three hundred thousand tons by year approximately (FAO, 2016). However, the white spot syndrome virus in America Pacific coast provoked a big economically lost in this industry. This situation generated a diversification of aquaculture in several countries, and Ecuador added the Tilapia as the second aquaculture species for exportation. This species could adapt in different ecosystems easily and sustainably production main depends on the quality and quantity of fry production. Some environmental, nutritional, and social factors significantly affect the hormonal processes in tilapia that further affect the quality and quantity oocytes during spawning process (Gowaty, 1994; Kellogg *et al*., 1995). In this sense, primarily the control of reproduction in fish culture is focused on the regulation of circulating hormones such as follicular stimulating hormone (FSH) and luteinizing hormone (LH) and subsequent action on a target tissue, generating profound biochemical changes. In ovarian cells of adult females, this action leads to the synthesis and secretion of circulating steroids such as 17β-estradiol (E2) consequently, increased synthesis of vitellogenin (Vtg), an essential precursor for the formation of oocytes, ovarian growth, and steroid-genesis in oviparous species (Samaee *et al*., 2009; Shappell *et al*., 2010; Tortolero *et al*., 2010; Dammann *et al*., 2011).

Several studies in tilapia showed that some strategies for endocrine regulation and ovulation failed and the response was poor with a limited number of

Corresponding editor: Alvaro Bicudo

spawns per cycle and low fertility (Pullin, 1987; Srisakultiew, 1993; Boonet *et al*., 2007). Moreover, these methods coupled with the use of light affects the quality of oocytes, with some changes in biochemical molecules like cyclooxygenase (COX) and prostaglandin E2 (PGE2). PGE2 is the result of the transformation of arachidonic acid (AA 20: 4, n-6) by the action of prostaglandin G/H synthase (PGG/H), previously released from the cell membrane by phospholipase A2. The PGG/H synthase exhibits two different ways of catalytic activity COX and oxidative catalyst component of AA and its transformation to PGG2 and hydroxy-peroxidase acting in reducing PGG2 group 15 hydroperoxyl in PGH2, which is highly unstable and is transformed in prostacyclins and thromboxanes. In this context, ovulation and spawning are considered an element of stress-inflammatory during reproduction and the action of prostaglandins will have a major role in the quality of oocytes (Bayarri *et al*., 2002; Van-Anholt *et al*., 2003; Boonet *et al*., 2007; El Sayed & Kawanna, 2007; Tsadiq, 2008; Lubzens *et al*., 2010; Onumah & Wessels, 2010).

This research evaluated the effects of E2 concentration on circulating Vtg in plasma and related molecules such as COX and PGE2 in tilapia oocytes during the reproductive period. This information could be useful to improve the evaluation methods and obtain a good quality and quantity fry in the industrial farm in Ecuador.

MATERIALS AND METHODS

Fish sample and hormone treatment

Experimental evaluation was conducted under real production conditions in Guayas Province, Ecuador. Seventy-two adult tilapias (319 ± 38 g), in an equal ratio of male and female, were kept randomized into 9 tanks, containing 1500 L of water. Suitable rearing conditions such as water recirculation system, with a temperature of 28 ± 2°C and natural light were used.

Two different treatment groups and a control were maintained in triplicate. Twelve female fish were treated with an intraperitoneal injection of estradiol-synthetic (5 µg g^{-1} body weight) (Cayman Chemicals Company, MI, USA) dissolved in vegetable oil. The same proportions were used with tilapia pituitary extracts (TP). Before injection, the fish were anesthetized with eugenol (5 mg L^{-1}). Blood was collected weekly from the caudal artery using a syringe internally coated with heparin. Plasma was separated by centrifuging at 1500 g for 15 min and stored at -80°C until further use. Matured ovaries were dissected and frozen in liquid nitrogen at -80°C until COX activity and PGE2 levels were measured. Parameters such as gonad somatic index (GSI), relative fecundity (FR), oocytes weight, and volume were also measured.

Analysis of hormonal steroid E2 and PGE 2

Quantification of E2 levels from *in-vivo* studies was performed by enzyme-linked -immunoassay sorbent assay (ELISA) using the commercial kit (Cayman Chemicals Company, MI, USA). ELISA was performed for all samples in duplicates and a separate standard curve was run for each ELISA plate. Different serial dilutions of the steroid were prepared and its levels were validated by running in parallel to the relevant standard curve. The limit of steroid detection was 20 pg mL^{-1}.

Ovary PGE2 levels were tested with prostaglandin E2 EIA kit Monoclonal antibody (Cayman Chemicals, USA). Samples (1 g of tissue) were homogenized in 5 mL of buffer (0.1 M PBS, pH 7.4, 1 mM EDTA and 10 µM indomethacin), and centrifuged at 8000 g. The supernatant was centrifuged at 3000 g for 10 min with ethanol and dried with nitrogen. The samples were acidified to pH 4.0 with the addition of 1 M acetate buffer and eluted in SPA filters (C-18). The purified fraction was dried with nitrogen and stored in phosphate buffered saline 1X (PBS) at -80°C for subsequent analysis by spectrophotometer.

COX activity

Cyclooxygenase total activity (COX1, 2) was tested with COX activity fluorescent kit (Cayman Chemicals, USA). The ovarian samples (1 g) were homogenized in 5 mL of cold buffer (100 mM Tris-HCl, pH 7.5, 1 mM of phenylmethylsulfonyl fluoride) in the tissue homogenizer at 50 oscillations/s, for 2 min, at 4°C. Prior to centrifugation at 10.000 g, the supernatant was removed. The analysis was performed using a fluorescence system test at 22°C, with a wavelength of excitation and emission of 530 nm and 590 nm, respectively. Resorufin standard was used, it is the product of the reaction between PGG2 and ADHP (10 -acetyl -3, 7 dihydroxy phenoxazine) and easily detectable under these conditions. Sheep´s COX 1, 2 were used as positive controls while COX inhibitors SC DUP-697 and -560 were used as negative controls.

Vitellogenin analysis

Plasma samples were treated with protease inhibitors (PMSF) and stored at -80°C for analysis. Vtg purification was performed on columns of quaternary ammonium anion-exchange Sortobind MA Q-15 (Sartorius, Germany) as mentioned by Shi *et al*. (2003). The protein content in the respective fractions was

further quantified, and confirmed by western blot, using polyclonal anti-Vtg sea bream and used in tilapia (Swart & Pool, 2009). The western blot analysis was used as a reference to locate the protein in SDS-PAGE, and subsequently calculated the relative concentration with a densitometry image analysis (image J free software).

Statistical analysis

The plasma E2 levels and Vtg levels were calculated for homogeneity of variance and normality with Levene and Shapiro-Wilk test and analyzed by one-way ANOVA. PGE2 levels were analyzed by Kruskal-Wallis nonparametric tests followed by Mann-Whitney test. Results were considered significant if $P \leq 0.05$. Calculations were performed using the InfoStat statistical package and graphic analyzer Graph Pad Prism 5. The data were expressed as mean ± SE.

RESULTS

Vitellogenin purification and detection by SDS PAGE and western blots

The purified Vtg obtained by quaternary ammonium anion exchange and mentioned above was confirmed by SDS-PAGE in different dilutions of purified Vtg (columns 1-5). The Vtg molecular weight was 170 to 200 kDa with a concentration about 1.13 mg mL^{-1} (Fig. 1).

General protein profile in SDS-PAGE revealed the presence and absence of bands between 130-210 kDa in both male and female tilapia samples. Notably, the effect of synthetic estradiol in males generates high levels of circulating Vtg with molecular weights between 130-170 kDa (Fig. 2).

The Western blot confirmed the presence of Vtg by strongly immunoreactive Rabbit anti seabream Vtg antibody visible as a major band of 170 kDa, which presumably represents a complete tilapia Vtg (Fig. 3).

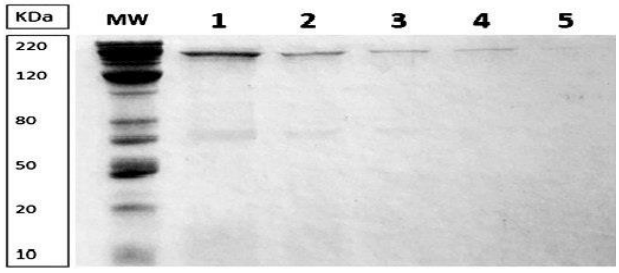

Figure 1. SDS-PAGE analysis of protein fractions. MW: molecular weight marker, 1: purified Vtg (concentration 1.127 mg mL^{-1}), 2: 563 µg mL^{-1}, 3: 281 µg mL^{-1}, 4:140 µg mL^{-1}, 5: 70 µg mL^{-1}.

Quantification of E2 and Vtg during the reproductive cycle of *Oreochromis niloticus*

Plasma E2 levels during reproduction period show significant differences between days (n = 36; P = 0.0002). This variation ranges from 7.7 to 22.9 ng mL^{-1} in the first five days, keeping concentrations of 9.7 to 20.8 ng mL^{-1} during the next 20 days (n =36; $P < 0.014$) (Fig. 4a).

However, Vtg behavior is different between treatments (n = 36; $P < 0.05$), in which exogenous and endogenous inducers generated a gradual and proportional increase in reproduction time (Fig. 4b). There is an interaction between E2 and Vtg (n = 36; P = 0.018) with an $R^2 = 0.92$. The intra-assay coefficient of variation (CV) was 6.2%, which was the measure of replicates of E2 samples within the assay. Similarly, the inter-assay CV was found to be 9.3% that is calculated by measuring replicates of the same sample in different assays.

Reproductive parameters

Twenty-five days after treatments ovarian tilapia female showed many changes. The GSI parameter was increased from 2.4 to 2.8% in ES and 2.4 to 3.5% in TP treatments (Table 1). Similarly, TP treatment showed 15.7 oocytes g^{-1} of female, with significant differences (n = 36; $P < 0.005$). The parameters such as oocyte volume and symmetry analysis showed significant differences (n = 200; $P < 0.0009$). The PT and ES treatments showed a higher number of mature oocytes (diameter >2 mm) and better symmetric form (Fig. 5).

COX activity and PGE 2 concentration

COX activity detected significantly differences between treatments (n = 200; P = 0.024), however the PGE2 concentrations did not show statistically significant differences (n = 200; P = 0.78). The medians of each treatment were between 0.07 to 1.68 pg mg^{-1}. The PGE2 concentration increased in control and estradiol treatments with 0.50 and 0.34 pg mg^{-1} respectively. Pituitary extract treatment maintained 0.29 pg mg^{-1} oocyte tissue (Fig. 6).

DISCUSSION

The study detected differences in circulating E2 levels after spawning and before ovulation period. This situation relates to the reproductive cycle of tilapia, where there is a parallel fluctuation of FSH β and LH gene expression after spawning and ovulation. This suggests that FSH and LH hormone levels are playing an important role in vitellogenesis, maturation, and ovulation in asynchronous fish. Therefore, plasma estra-

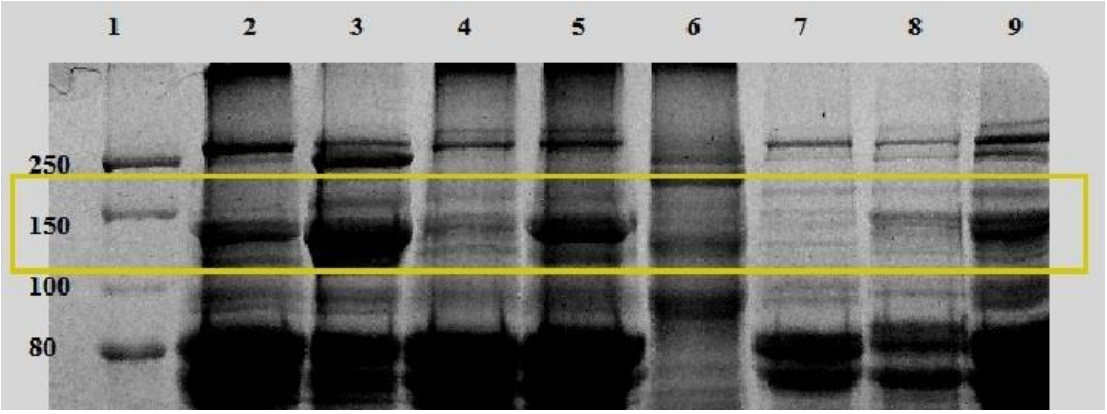

Figure 2. SDS-PAGE 8%: 10-250 kDa protein marker (lane 1), samples tested included female plasma (lane 2 to 5), human plasma as a control (lane 6), tilapia male plasma (lane 7), tilapia male plasma treated with pituitary extract (lane 8), tilapia male plasma treated with pure estradiol (lane 9).

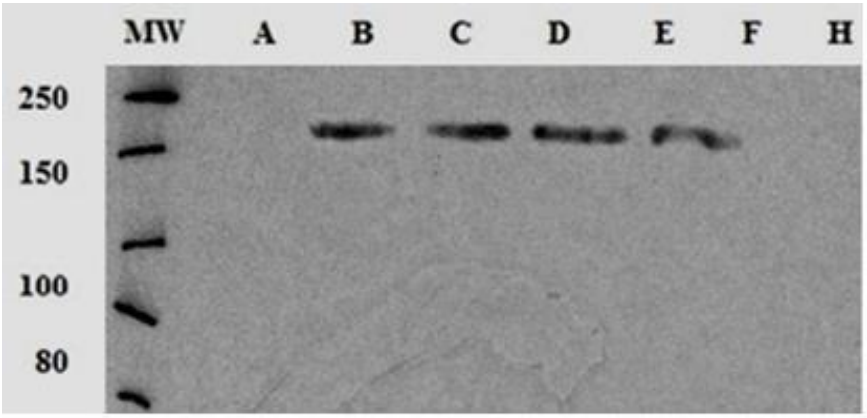

Figure 3. Western blot control sample: 10-250 kDa protein marker (lane MW), female plasma day 1 after spawning (lane A), female plasma day 5 (lane B), female plasma day 10 (lane C), female plasma day 15 (lane D), female plasma day 20 (lane E), female plasma day 25 (lane F), tilapia male plasma (lane H).

diol levels have an effect not only on the variations in Vtg concentrations but also on the concentrations of other molecules as prostaglandins, which have an intimate action on ovulation and spawning (Levavi *et al.*, 2006, 2010; Aizen *et al*., 2007; Nigel & Fyhn, 2010).

Furthermore, the experiment showed different Vtg concentration during the reproductive time. The differences between treatments noted in the first five days, and concentrations of Vtg gradual increasing during the next ten days. This behavior differs from control treatment, which implies a change in the normal hormonal process of this species. When hormones are released in a pulsatile form, the signal will be continuous. On the contrary, when the hormones are released continuously the target cell will be saturated, and the receptors desensitize. In addition, the system improves hormonal process after the tenth day, increasing the vitellogenin levels, decreasing E2 concentrations and stimulated the ovarian growth. This induced the proportionality of stages in the groups of oocytes in the ovaries. This implies that circulating Vtg levels regulate circulating 17β estradiol levels and reciprocally through simultaneous activation Vtg genes and inhibition of Growth Hormone-Insulin-Like Growth Factor 1 (Davis *et al.*, 2007). Some environmental impact studies in salmons as *Salvelinus alpinus* showed different response under an action of estrogens generating different amounts of Vtg (Berg *et al*., 2004).

In the other hand, the concentration of circulating hormone also depends on the number cellular receptors present on the cell and each cell regulates its own receiver for a given response (Reis-Henriques *et al*., 1997). Relative fecundity variable showed significant

Table 1. Reproductive parameters of an adult female of *Oreochromis niloticus* during reproductive cycle after ES and TP treatments. Different superscripts within rows indicate significant differences (n = 36; $P < 0.05$). An absence of superscripts implies that there no significant differences ($P > 0.05$). The results are expressed as mean ± SD.

Parameters	Control	Estradiol	Pituitary
GSI (%)	2.4 ± 0.22	2.8 ± 0.29	3.5 ± 0.62
Oocyte (mg)	3.0 ± 0.99	2.8 ± 1.44	2.7 ± 1.42
Oocyte (mm^3)	19.1 ± 12.00	20.5 ± 8.14	22.8 ± 6.20
Oocytes /g female	8.1 ± 3.27[a]	12.5 ± 6.68[ab]	15.7 ± 5.78[b]

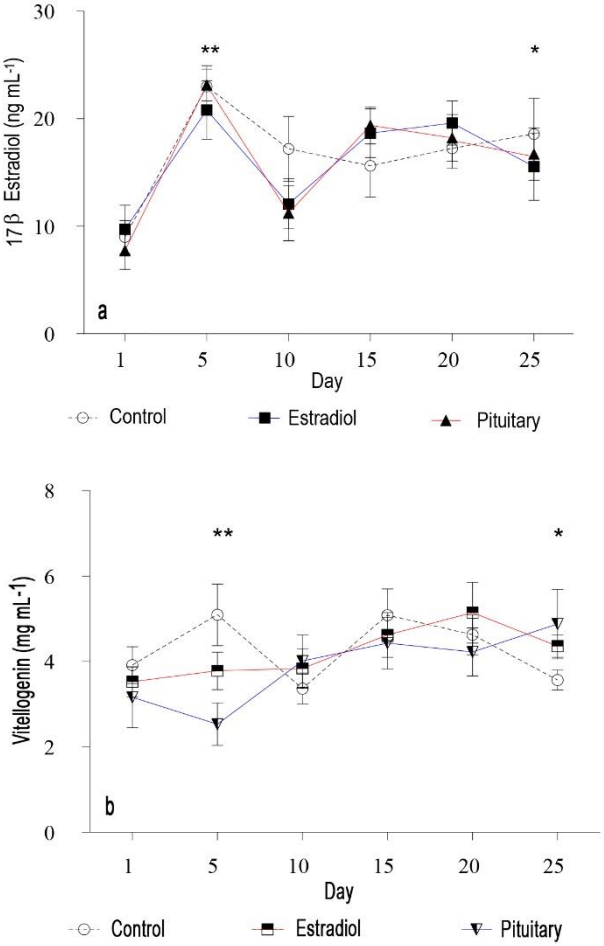

Figure 4. a) Plasma estradiol and b) vitellogenin levels in adult females of *Oreochromis niloticus* under three different experimental conditions: Pure estradiol, pituitary extract, and control. The results are expressed as mean ± ES, whereas, n= 36. $^{**}P = 0.001$; $^{*}P = 0.01$.

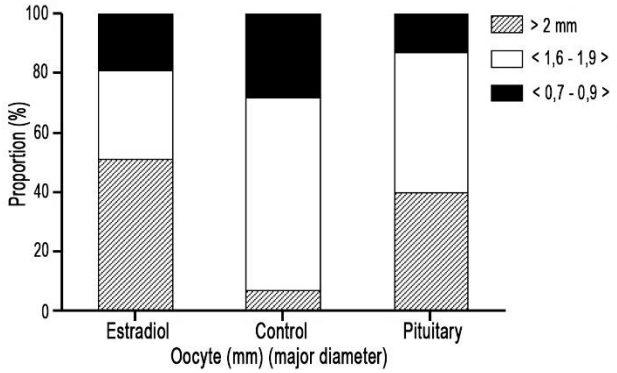

Figure 5. The number of oocytes and the parameters such as the diameter of the major axis (mm) and stage of development. For pre vitellogenic <0.7-0.9 mm>; for vitellogenic <1.6-1.9 mm>; for mature oocyte it was> 2 mm. The results are expressed as proportion (%).

differences between treatments. In the industrial fish farm, the normal oocytes matured range is 1.3 to 7.2 eggs g^{-1} female (Rhida & Cruz, 2000; El-Sayed & Kawanna, 2007; Ponzoni *et al.*, 2011). The results obtained by ES and TP treatments lead to double the capacity of the control and improve the number of embryos in the production process. In this sense, the oocytes volume and it symmetry analysis showed significant differences between control and treatment groups ($P < 0.0009$). Also noted that treatment with ES and PT produces a higher number of matured oocytes (diameter > 2 mm) and symmetrically better in shape than its early stages (diameter <1.6-1.9 mm). Mendoza *et al.* (2011), detected four stages of oocyte development in *Oreochromis niloticus* related to the diameter of the oocyte. Diameters between 0.1 and 1.6 mm correspond to previtellogenic and vitellogenic stages, while diameters between 2 and 3 mm indicate the stages of maturity and spawning. Cabrita *et al.* (2009) also detected mature oocyte size between 2.4-2.5 mm in *Oreochromis niloticus*, mentioning that this feature would be given by age and type of food. Furthermore, Nagahama & Yamashita (2008) and Carrillo (2009) defined that the endocytosis of vitellogenin and other compounds in the retention period of meiosis in prophase-I decided the oocyte growth and after attaining maximum growth the meiosis to metaphase II restarts.

The COX activity and PGE2 levels in tilapia oocytes had changed and were inhibited by the action of ES and PT. The oocytes quality could be affected because these molecules are a functional component for

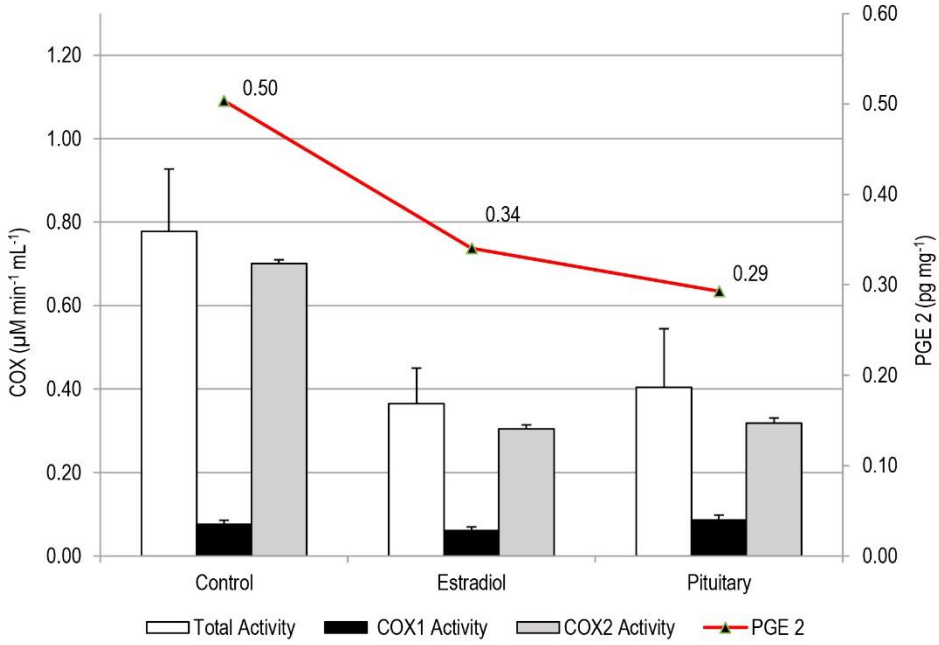

Figure 6. Total COX Activity and PGE 2 in tilapia ovaries by different treatments: control, estradiol and pituitary extract. The data were expressed as mean ± ES.

ovulation and spawn and have a close interaction with membrane receptors in embryogenesis stage (Kellner & Van Der Kraak, 1993; Van Aanholt *et al*., 2003; Bonnet *et al.*, 2007; Lister & Van Der Kraak, 2009).

In conclusion, the dynamic levels of E2 and Vtg secretion, it changed under the inducers action, generating a change in the Vtg circulating levels after tenth days of the reproductive cycle of tilapia. This behavior could improve important productive parameters but also, it can inhibit some main molecules which are related to ovulation and spawn.

ACKNOWLEDGEMENTS

This research was supported by funding from the Universidad de las Fuerzas Armadas -ESPE and the authors would like to thank, Dr. Claudia Segovia and Dr. Francisco Flores for their critical review of this manuscript. The co-author (Dr. Bangeppagari-Manjunatha) greatly acknowledges the Korea Research Fellowship Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning.

REFERENCES

Aizen, J., H. Kasuto, G. Matan, H. Zakay & B. Levavi-Sivan. 2007. Tilapia Follicle-Stimulating Hormone (FSH): immunochemistry, stimulation by gonadotropin-releasing hormone, and effect of biologically active recombinant FSH on steroid secretion. Biol. Reprod., 76: 692-700.

Bayarri, M., A. Madrid & J. Sanchez-Vazquez. 2002. Influence of light intensity, spectrum, and orientation on sea bass plasma and ocular melatonin. J. Pineal Res., 32: 34-40.

Berg, H., C. Moding & P. Olsson. 2004. 17 β-estradiol-induced vitellogenesis is inhibited by cortisol at the post-transcriptional level in Arctic char (*Salvelinus alpines*). Reprod. Biol. Endocr., 2: 62-72.

Bonnet, E., A. Fostier & J. Bobe. 2007. Microarray-based analysis of fish egg quality after natural or controlled ovulation. BMC Genomics, 8: 55-63.

Cabrita, E., V. Robles & P. Herraez. 2009. Methods in reproductive aquaculture marine and freshwater species. Taylor and Francis Group, New York, 579 pp.

Carrillo, M. 2009. La Reproducción de los peces: aspectos básicos y sus aplicaciones en acuicultura. Cerebro y reproducción en peces. Bases neurales y neuroendocrinas. Fundación Observatorio Español para la Acuicultura, Madrid, 699 pp.

Dammann, A., N. Shappell, S. Bartell & H. Schoenfussa. 2011. Comparing biological effects and potencies of estrone and 17 β-estradiol in mature fathead minnows, *Pimephales promelas.* Aquat. Toxicol., 105: 559-568.

Davis, L., N. Hiramatsu, K. Hiramatsu, B. Reading, T. Matsubara, A. Hara, C. Sullivan, A. Pierce, T. Hirano & G. Grau. 2007. Induction of three vitellogenins by 17 beta-estradiol with concurrent inhibition of the

growth hormone-insulin-like growth factor 1 axis in a euryhaline teleost, the Tilapia (*Oreochromis mossambicus*). Biol. Reprod., 77: 614-625.

El-Sayed, A.F.M. & M. Kawanna. 2007. Effects of photoperiod on growth and spawning efficiency of Nile tilapia (*Oreochromis niloticus L.)* broodstock in a recycling system. Aquacult. Res., 38(12): 1242-1247.

Food and Agriculture Organization of the United Nations (FAO) 2016. Statistic at FAO citations of Internet sources. [http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en]. Reviewed: 12 February 2016.

Gowaty, P.A. 1994. Architects of sperm competition. Trends Ecol. Evol., 9: 160-162 pp.

Kellner, R. & G. Van Der Kraak. 1993. Multifactorial regulation of prostaglandin synthesis in preovulatory goldfish ovarian follicles. Biol. Reprod., 46: 630-635.

Kellogg, K.A., J.A. Markert, J.R. Stauffer & T.D. Kocher. 1995. Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. Proc. R. Soc. Lond. B, 260: 79-84.

Levavi, B., J. Biran & E. Fireman. 2006. Sex steroids are involved in the regulation of gonadotropin-releasing hormone and dopamine D2 receptors in female tilapia pituitary. Biol. Reprod., 75: 642-650.

Levavi-Sivan, B., J. Borged, E. Mañanos, A. Gómez & J. Lareyre. 2010. Perspectives on fish gonadotropins and their receptors. Gen. Comp. Endocr., 165: 412- 437.

Lister, A. & G. Van Der Kraak. 2009. Regulation of prostaglandin synthesis in ovaries of sexually-mature zebrafish (*Danio rerio*). Mol. Reprod. Dev., 76: 1064-1075.

Lubzens, E., Y. Graham, J. Bobe & J. Cerda. 2010. Oogenesis in teleosts: how fish eggs are formed. Gen. Comp. Endocr., 165: 367-389.

Mendoza, B., J. Gómez-Marquez & G. García- Alberto. 2011. Ciclo reproductor e histología de las gónadas de *Oreochromis niloticus*. Cienc. Pesq., 19(2): 23-36.

Nagahama, Y. & M. Yamashita. 2008. Regulation of oocyte maturation in fish. Dev. Growth Differ., 50: S195-S219.

Nigel, R. & H. Fyhn. 2010. Requirement for amino acids in the ontogeny of fish. Aquacult. Res., 41: 684-716.

Onumah, E.E. & S. Wessels. 2010. Stocking density and photoperiod manipulation in relation to estradiol profile to enhance spawning activity in female Nile tilapia. Turkey J. Fish. Aquat. Sci., 10(4): 463-470.

Ponzoni, R.W, N.H. Nguyen, H.L. Khaw, A. Hamzah, K.R.A. Bakar & H.Y. Pee. 2011. Genetic improvement of Nile tilapia (*Oreochromis niloticus*) with special reference to the work conducted by the World Fish Center with the GIFT strain. Rev. Aquacult., 3: 27-41.

Pullin, E. 1987. Synchronous spawning of Nile tilapia through hypophysation and temperature manipulation. Ponencia presentada en el II Simposio Internacional de Acuacultura, Bangkok, pp. 259 -275.

Reis-Henriques, M., M. Cruz & J. Pereira. 1997. The modulating effect of vitellogenin on the synthesis of 17 β estradiol by rainbow trout (*Oncorhynchus mykiss*) ovary. Fish Phys. Biochem., 16: 181-186.

Ridha, M.T. & E.M. Cruz. 2000. Effect of light intensity and photoperiod on Nile tilapia *Oreochromis niloticus*. Aquacult. Res., 31: 609-617.

Samaee, S., F. Lahnsteiner, G. Gimenez, A. Estévez, B. Sarg & H. Linder. 2009. Quantitative composition of vitellogenin-derived yolk proteins and their effects on the viability of embryos and larvae of common dentex (*Dentex dentex*), a marine Pelagophil teleost. J. Exp. Zool. A, 311: 504-520.

Shapell, N., K. Hyndman, S. Bartell & H. Schoenfuss. 2010. Comparative biological effects and potency of 17 alfa- and 17 beta-estradiol in fathead minnows. Aquat. Toxicol., 100: 1-8.

Shi, G., J. Shao, G. Jiang, Q. Wang, Y. Lu, J. Liu & J. Liu. 2003. Membrane chromatographic method for the rapid purification of vitellogenin from fish plasma. J. Chromatogr. B, 785: 361-368.

Srisakultiew, P. 1993. Studies on the reproductive biology of *Oreochromis niloticushesis* L. Ph.D. Thesis, University of Stirling, Scotland, 267 pp.

Swart, J.C. & E.J. Pool. 2009. The development and validation of a quantitative ELISA for *in vivo* and *in vitro* synthesized vitellogenin from Mossambicus tilapia (*Oreochromis mossambicus*), J. Immunoass. Immunoch., 30(2): 208-223.

Tortolero, D., R. Campos-Ramos, M. Burgos-Aceves, J. Urbiola & G. Durán. 2010. Effects of compressed seasonally changing day-length cycles on spawning performance, production of viable eggs and levels of vitellogenin in plasma in female yellowtail snapper *Lutjanus argentiventris*. J. Fish Biol., 77: 2285-2297.

Tsadik, G. 2008. Effects of maternal age on fecundity, spawning interval, and egg quality of Nile tilapia, *Oreochromis niloticus* (L.). J. World Aquacult. Soc., 39(5): 671-677.

Van Anholt, R.D., T. Spanings, W. Koven & S.E. Wendelaar-Bonga. 2003. Effects of acetylsalicylic acid treatment on thyroid hormones, prolactin, and stress response of tilapia (*Oreochromis mossambicus*). Am. J. Physiol. Reg. I, 285: 1098-1106.

Received: 29 August 2016; Accepted: 24 May 2017