Zagazig J. Agric. Res., Vol. 43 No. (4) 2016
http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

INDUCTION OF OVULATION IN *Liza ramada* (RISSO, 1826) OUT OF SEASON

Mohamed A.E. Naiel*, A.A. El-Darawany, A.E. Nasr and Kh.M. Al-Marakby

Anim. Prod. Dept., Fac. Agric., Zagazig Univ., Egypt

ABSTRACT

The present work was designed to determine the suitable hormonal requirements for induction of ovulation in the thin-lipped grey mullet (*Liza ramada*) reared in brackish water earthen pond out of season. On April 2014, eight groups of grey mullet (6 females / group) were collected and kept in aquaculture pond. The first group (G_1) did not receive the hormonal treatment. The mullets of the other seven groups were subjected to intramuscular injection of hormones as follows: G_2, 4500 IU/Kg human chorionic gonadotropin (hCG); G_3, 3 mg melatonin (ML) /fish; G_4, 3 carp pituitary extract (cPE) / fish; G_5, a combination of 4500 IU/Kg hCG + 3 cPE / fish; G_6, a combination of 4500 IU/Kg hCG + 3mg/ ML fish; G_7 , a combination of 4500 IU/Kg hCG + 3 adrenal gland (AD) / fish; G_8, a combination of 3 cPE + 3mg/ ML fish. The results after 24 hr., of injection revealed that estradiol 17-β (E_2) level in the collected serum experimental mullets was increased ($P < 0.05$) with G_2 treatment. In the same trend, gonadosomatic index (GSI) was significantly ($P < 0.05$) increased with G_2. The serum Progesterone (P) concentration and Progesterone/Estradiol 17-β ratio (P/E_2) were significantly ($P < 0.05$) increased with G_5 than other groups. The G_5 group showed oocytes maturation and ovulation superior to that of all other groups, with ripped oocyte and other different maturity stages in *Liza ramada* out of season. It could be concluded that, 4500 IU/kg of hCG and 3 cPE combination had successfully induced the oocyte maturation in *Liza ramada* fish reared in brackish water under captivity out of season and a higher dose would induce the ovulation and spawning.

Key words: hCG, cPE, adrenal gland, ML, grey mullet.

INTRODUCTION

Mugilidae is one of the popular and fast growing fishes being cultured in tropical and subtropical regions (Kumar *et al.,* 2015). The thin-lipped grey mullet, *Liza ramada* is an important and attractive species for farming in marine, brackish and fresh water.

The collection of *Liza ramada* mature females is not possible from the natural habitat since the pre-spawning females would have then migrated to the sea for spawning (Mousa and Mousa, 2003). In Egypt, the aquaculture of family mugilidae depended on natural fry collection, which cannot satisfy the increasing demand for the juveniles. The possibility of inducing and synchronizing spawning can be very useful to facilitate fish farming, particularly in species that achieve ovarian development in captivity without ovulation (Mousa, 2010). In order to meet this great demand, controlled spawning of this fish and artificial rearing of the fry are the best means for fish seed production to meet the large need of the fish farmers. Furthermore, controlled reproduction of the fish may also make it possible to develop method for selective breeding of superior strains and for furnishing parasite- and disease-free fish seeds (Tang, 1964).

Under captive conditions, mullet brooders do not spawn spontaneously, with both genders displaying reproductive dysfunctions, even in full strength seawater (Aizen *et al.,* 2005). However, Hormonal treatments are widely used

***Corresponding author: Tel. :** +201113992455
E-mail address: mohammednaiel.1984@gmail.com

to stimulate reproductive processes and induce spawning fish that will not spawn in captivity on their own (Bui *et al.*, 2010).

The aim of the present study is to induce ova maturation and ovulation in *Liza ramada* out of season by different hormonal treatments at brackish water earthen ponds.

MATERIALS AND METHODS

Broodstock Management

Mature females of *Liza ramada* were reared at Shalatayat culture ponds in Port Said. The fish were collected during the spawning season (December 2013). They were 3 years old with weight ranged from 369 to 511 g and total length ranged from 32 to 35 cm. They were reared in outdoor earthen pond at Sahl El-Hussinia (30°57'26"N, 32°4'3"E) aquaculture farm. On April 2014, forty eight females (six per each treatment) were allotted into eight partitions (5x5x1 meter (m) per each, 25 m^3) separated by nets. Water quality parameters were as follows: water temperature was ranged from 22 to 32°C, dissolved oxygen content was 5 mg O_2/liter (l), total nitrogen as NH_4Cl was ranged between 3.4 to 5.1 mg/l, total alkalinity 690-700 mg/l, total hardness 30-37 mg/l, total dissolved solids 6.20-6.59 ppt, electric conductivity (EC) 17.9 - 23.3 mV and pH value was 8.07. The light regime was that of natural photoperiod (13 hours).

Hormonal Used and its Resources

The used hCG is Pregnyl, Nile Company for Pharmaceuticals, Cairo, Egypt. Melatonin prepared by dissolving one tablet of melatonin 3 mg in 1 ml saline solution. The used melatonin tablets are manufactured by NATROL, Inc. Chatsworth, CA 91311 USA. The adrenal gland extraction was prepared by extracting three adrenal glands according to Bern (1967) in 1 ml saline solution. cPE prepared by dissolving three cPG in 1 ml saline solution.

Experimental Design and Hormone Strategies Injection

The females were divided into eight groups. The first one was the control group (G_1), while the other seven groups subjected to the different hormonal injections. The G_2 of females was injected with human chorionic gonadotropin (hCG), 4500 IU/Kg. The $G_3$was injected with 3 mg melatonin / fish. The $G_4$was injected with an extract of three carp pituitary glands / fish.

In the second injection strategy fish was injected with a combination of two components, as in the G_5 injected with a combination of 4500 IU/Kg hCG and an extract of three carp pituitary glands / fish. The G_6 group was injected with a combination of 4500 IU/Kg hCG + 3 mg melatonin / fish. While, the G_7 was injected with a combination of hCG at the same dose and an extraction of three adrenal gland / fish. The G_8 was injected with a combination of an extract of three carp pituitary glands + 3 mg melatonin / fish.

In all cases, the fishes received the hormonal treatments at 08.00 AM by injection into the dorsal muscular after anesthesia with MS222.

Blood Sampling and Hormonal Assay

After 24 hr of the hormonal injection, Blood samples were collected from the caudal vein of all females and centrifuged at 3000 rpm for 15 minutes. Serum kept at - 20°C until analysis. Oestradiol 17-β and progesterone concentrations in the sera were determined using radioimmunoassay kits produced by Diagnostic Product Corporation (Los Angeles) at the Lab. of Middle Eastern Regional Radioisotopes Center for the Arab Countries (MERRCAC).

Measurement of Body and Gonad Parameters

Fish samples were transported to the laboratory for the measurement of total length (TL), standard length (SL) to the nearest 0.1 millimeter (mm) and body weight (W) to the nearest 0.1 g.

Gonadosomatic index (GSI) was calculated according to Render *et al.* (1995) from the following equation:

GSI = Gonad weight / (body weight - gonad weight) × 100

Histological Examination

The ovary specimens were quickly collected 24 hrs., post-injection, weighed and fixed overnight in 4% paraformaldehyde. Then, the

tissues were dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin. Tissue sections (3 μm thick) were obtained using rotary microtomes then deparaffinized and stained with hematoxylin and eosin at Laboratory of Anatomy, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan.

Statistical Analysis

Assessment of the results was performed using the one-way analysis of variance (ANOVA) procedure followed by Duncan's Multiple Range test (Duncan, 1955) with SPSS (2012) version 19. Data were expressed as means ± S.E. The 0.05 level of probability was used as the criterion for significance.

RESULTS

GSI and Serum Steroid Hormones

It is obvious that the GSI and Estradiol-17β (E_2) values were significantly increased ($P<0.05$) in G_2 (Figs. 1 and 2).

On the other hand, the level of progesterone hormone and the ratio progesterone estradiol-17β (P/E) ratio were significantly increased ($P<0.05$) with G_5 treatment (Figs. 2 and 3).

Microscopic Characteristics of the Ovary

The ovary showed different maturation stages of oogenetic development (Fig. 4,A,B,C).

Oogonia

There are smallest germ cells, found either in clusters or solitary. It is round to spherical shaped, characterized by weak basophilic cytoplasm and large light nucleus (Fig. 4 B).

Chromatin nucleolus stage

The oocytes are small spherical to polygonal cells with strongly basophilic homogenous cytoplasm. (Fig. 4 B).

Perinucleolar stage

It is characterized by two stage. Early perinuclear stage; the oocyte increase in the size. The nucleus is spherical, large, occupies greater part of the cytoplasm, lightly stained and contains 1-2 peripherally located nucleoli (Fig. 4. B).

Late perinuclear stage; the oocyte increase in the size and characterized by the appearance of one or few small vacuoles in the cytoplasm. The nucleoli increase in number nucleus contains 1-2 peripherally located (Fig. 4. B).

Previtellogenic and vitellogenic oocytes (Primary yolk stage or yolk vesicle (cortical alveoli) stage

Its characterized by increase in the size of oocyte which appear ovoidal in shape, weakly basophilic cytoplasm, and multiple nucleoli near to nuclear membrane with many vacuoles (Figs. 4 A). In more developed stages, the size of the vacuoles is increased; also yolk vesicles begin to be arranged into two distinct layers (one near the nuclear membrane and the other is near the oocyte membrane). Also, yolk globules of round to oval intensively acidophilic stain started to be formed secondary yolk stage.

Ripe oocyte

Characterized by the presence oval to pearl like shape, most of the vacuoles connected to each other and formed spaces between them in the cytoplasm, the nucleus began to loss its membrane and liberate its substance into the cytoplasm and disappear, some of the large yolk granules coalesced to form larger yolk drop that undergo liquefaction, some unovulated mature yolk eggs undergoining resorption (atresia) (Fig. 4 C).

According to these results, the G_5 group showed oocytes in different maturity stages where numerous oogonia in clusters appeared for further spawning with chromatin nucleolus stage (ch), few early perinuclear oocyte (Epo) and numerous late perinuclear oocyte, this in addition to many atretic and ripped oocytes appeared (R) (Fig. 4 C).

DISCUSSION

These results clearly illustrate the poor reproductive performance of the in Thin-lipped grey mullet (*Liza ramada*) capitative out of season and indicate possible means for its improvement. Thin-lipped grey mullet (*Liza ramada*) do not spawn spontaneously when reared

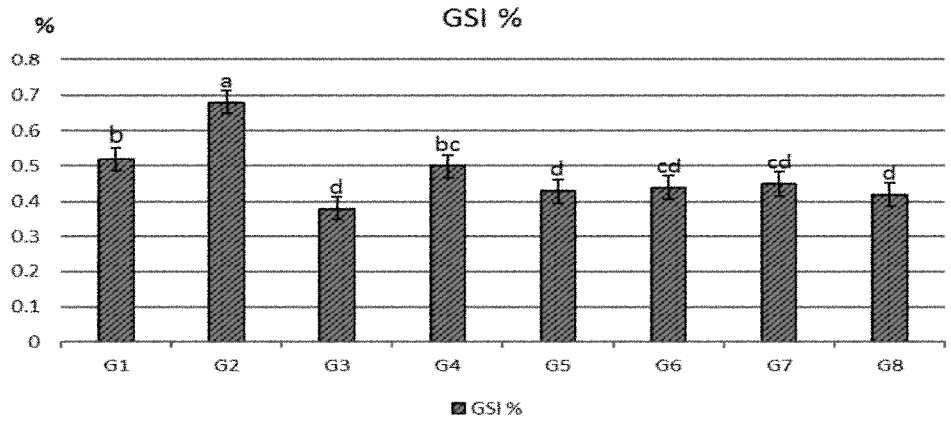

Fig. 1. Gonadosomatic index (GSI) values of *Liza ramada* 24 hr post injection with tested hormones. Each bar carrying different letters was significantly different ($p < 0.05$) (mean±S.E , n=3).

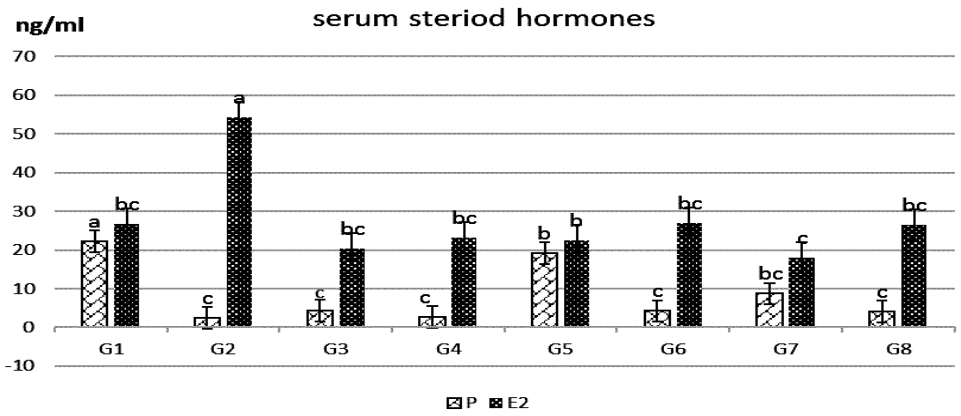

Fig. 2. Serum steroid hormones [Estradiol-17*β* (E2) and progesterone (P)] values of *Liza ramada* 24 hr post injection with tested hormones. Each bar carrying different letters was significantly different ($p < 0.05$) (mean±S.E , n =3).

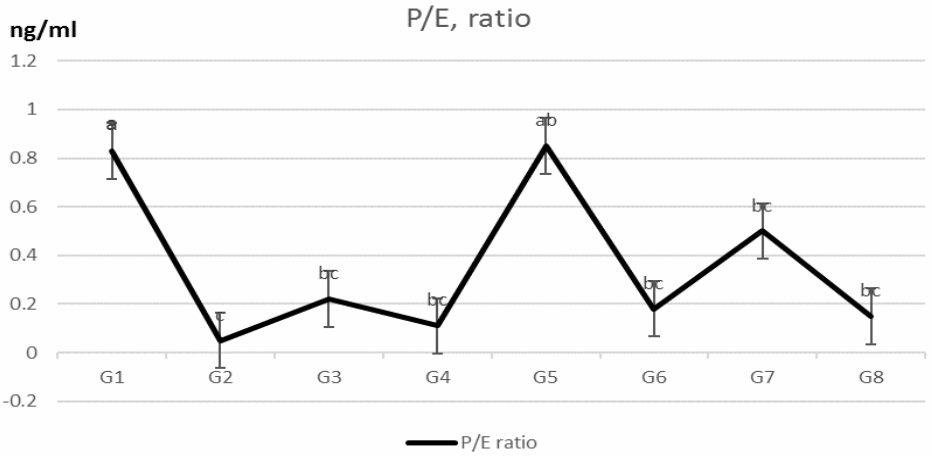

Fig.3. Progesterone / estradiol-17*β* (P/E) ratio of *Liza ramada* 24 hr post injection with tested hormones. The part of curve carrying different letters was significantly different ($p < 0.05$) (mean ± S.E , n=3).

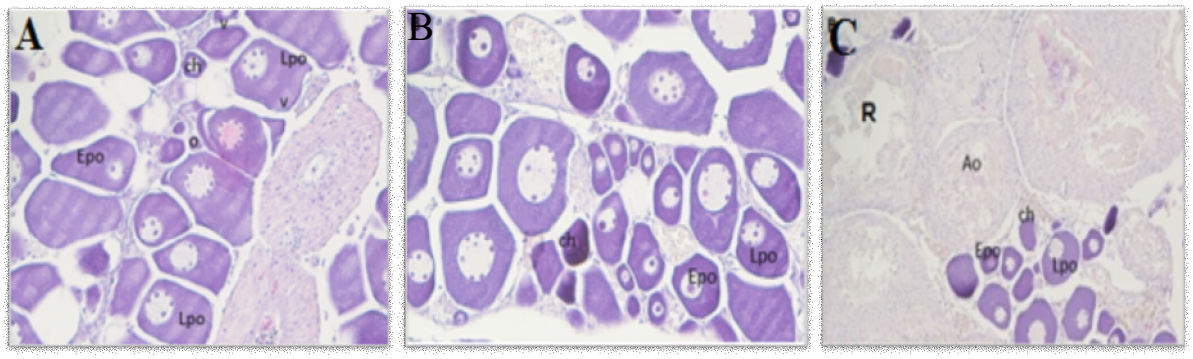

Fig. 4. Transverse section of the ovary H&E stained section (A&B), PAS stained section (C) : (A) treatments (G_3, G_4, G_6, G_7, G_8) showing oogonia (o), chromatin nucleolus stage (ch), few early perinuclear oocyte (Epo) and numerous late perinuclear oocyte (Lpo) with few vacuoles (v) : (B) treatments (G_1, G_2) showing oogonia (o), chromatin nucleolus stage (ch), few early perinuclear oocyte (Epo) and numerous late perinuclear oocyte (Lpo): (C) transverse section of the ovary (G_5), showing oocytes in different maturity stages; numerous oogonia in clusters (O) chromatin nucleolus stage (ch), few early perinuclear oocyte (Epo) and numerous late perinuclear oocyte (Lpo), many atretic oocytes (Ao), few ripe oocyte (R)

in captivity. The fish migrates to the sea to propagate in the spawning season. Induced breeding is necessary to control timing and synchrony of egg production (Elakkanai *et al.,* 2015). Few trials were done using hormone therapy for the induction of gonadal maturation and ovulation in fish with a limited success. In the present work, we examined the effectiveness of different types of hormones either individually or in combination to induce the ovaries maturation and ovulation out spawning season. Gonadal maturation in teleost fish is mainly regulated by the brain-pituitary-gonadal axis (Zohar and Mylonas, 2001). The role of pituitary gonadotropins in teleost gonadal maturation has been reported in eel, catfish and salmon (Levavi- Sivan *et al.*, 2010; Schulz *et al.*, 2010). Histological examination revealed that the combination of hCG and cPE in fish out of reproductive season, increase the final oocyte maturation and ovulation. Moreover, hCG alone significantly increased GSI compared with other groups fish indicating the increase in ovarian weight. Over the past years, hCG that acts directly on the level of the gonads (Zohar and Mylonas, 2001) has been increasingly used for the induction of spawning in many fish species. Ovarian gonadosomatic index and oocyte development is associated with changes in E_2 level in circulation (Lee and Yang, 2002). The high levels of E_2 in G_2 were accompanied with high gonadosomatic indices. Hence, as maturation of oocytes progressed in the ovary, levels of E_2 also increased in the plasma of *Liza ramada*, in spite of, G_2 was not the best treatment group. A significant, role of E_2 in oocyte maturation and vitellogenesis was detected. Sex steroids in female fish perform major roles in oocyte maturation, ovulation and spawning. The Synthesis of vitellogenin and the increase of the ovarian size during final oocyte maturation are controlled by E_2. Estradiol 17-β is directly related to gonadosomatic index (Sabet *et al.,* 2009; Coccia *et al.,* 2010). On the other hand, in G_7, cortisol plays important regulatory roles during reproduction, including the regulation of final oocyte (Cook *et al.*, 1980), ovulation (Hirose and Ishida, 1974), spawning behavior (Bry, 1985), and the mobilization of energy stores required for reproduction (Greenberg and Wingfield, 1987). In contrast, cortisol has deleterious effects on reproduction *via* reducing normal sex steroid production, pituitary gonadotropin content and gamete quality (Campbell *et al.* 1992; Carragher and Sumpter, 1990). The involvement of the hypothalamus and the pituitary gland in the control of cortisol secretion has been well established in fish with CRF and ACTH as the most important secretagogues and the interrenal

end products of the brain-pituitary- interrenal axis (Wendelaar, 1997). There is also histological evidence supporting the involoment of ACTH in response to stressors in these animals (Donaldson, 1981; Mousa and Mousa, 1999).

Lastly, histologically the oocyte maturation showed the following phases at the treated groups: first growth phase which includes chromatin stage, early and late perinucleolus stage. Second growth phase includes vacuolization stage, yolk stages (primary, secondary and titatry), mature stage and ripe stage than in control group.

The obtained results cleared that, 4500 IU/kg of hCG and 3 cPE combination had successfully induced the oocyte maturation in *Liza ramada* fish reared in brackish water under captivity out of season and a higher dose would induce the ovulation and spawning.

Acknowledgments

We are grateful to Dr. Yaser Elewa, Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt, for his assistance in the description of histological sections of ovaries.

REFERENCES

Aizen, J., I. Meiri, I. Tzchori, B. Levavi-Sivan and H. Rosenfelda (2005). Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. General and Comparative Endocrinol., 142: 212–221.

Bern, H. A. (1967). Hormones and endocrine glands of fishes studies of fish endocrinology reveal major physiologic and evolutionary problems. Science, 158(3800), 455-462.

Bry, C. (1985). Plasma cortisol levels of female rainbow trout (*Salmo gairdneri*) at the end of the reproductive cycle: relationship with oocyte stages. Gen. Comp. Endocrinol., 57 : 47–52.

Bui, T.M., L.T. Phan, B.A. Ingram, T.T.T. Nguyen, G.J. Gooley, H.V. Nguyen, P.T. Nguyen and S.S. De Silva (2010). Seed production practices of striped catfish, Pangasianodon hypophthalmus in the Mekong Delta region, Vietnam. Aquac., 306 : 92–100.

Campbell, P.M., T.J. Pottinger and J.P. Sumpter (1992). Stress reduces the quality of gametes produced by rainbow trout. Biol. Rep., 47: 1140 – 1150.

Carragher, J.F. and J.P. Sumpter (1990). The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. Gen. Comp. Endocrinol., 77: 403–407.

Coccia, E., E. De Lisa, C. Di Cristo, A. Di Cosmo and M. Paolucci (2010). Effects of estradiol and progesterone on the reproduction of the freshwater crayfish *Cherax albidus*. Biol Bull., 218:36–47

Cook, A.F., N.E. Stacey and R.E. Peter (1980). Periovulatory changes in serum cortisol levels in the goldfish, *Carassius auratus*. Gen. Comp. Endocrinol., 40: 507–510.

Donaldson, E.M. (1981). The pituitary-interrenal axis as an indicator of stress in fish. In: Pickering, A.D. (Ed.), Stress in Fish. Acad. Press, London, 11–47.

Duncan, D.B. (1955). Multiple Range and Multiple F-test. Biometrics, 11:1-42.

Elakkanai, P., T. Francis, B. Ahilan, P. Jawahar, P. Padmavathy, N. Jayakumar and A. Subburaj (2015). Role of GnRH, hCG and kisspeptin on reproduction of fishes. Indian J. Sci. and Technol., 8 (17): 1-10.

Greenberg, N. and J.C. Wingfield (1987). Stress and reproduction: reciprocal relationships. In: Norris, D.O., Jones, R.E. (Eds.), Hormones and Reproduction in Fishes, Amphibians and Reptiles. Plenum Press, New York, 461–505.

Hirose, K. and R. Ishida (1974). Effects of cortisol and human chorionic gonadotropin (HCG) on ovulation in ayu, *Plecoglossus altivelis*. J. Fish Biol., 6: 557–564.

Kumar, P., A.R.T. Arasu, M. Kailasam, K. Sukumarran, R. Subburj, G. Tyagraj and M. Natarajan (2015). Gonadal development and steroid hormone profile of wild caught grey mullet (*Mugil cephalus*). Biol. Rhythm Res., 46 (4) : 601-610.

Lee, W.K. and S.W. Yang (2002). Relationship between ovarian development and serum levels of gonadal steroid hormones, and induction of oocyte maturation and ovulation in the cultured female Korean spotted sea bass *Lateolabrax maculatus* (Jeom-nong-eo). Aquac., 207 (1): 169-183.

Levavi-Sivan, B., J. Bogerd, E. L. Mañanós, A. Gómez and J.J. Lareyre (2010). Perspectives on fish gonadotropins and their receptors. Gen. and Comp. Endocrinol., 165 (3): 412-437.

Mousa, M.A. (2010). Induced spawning and embryonic development of *Liza ramada* reared in freshwater ponds. Anim. Reprod. Sci., 119 : 115–122 .

Mousa, M.A. and S.A. Mousa (2003). Immunohistochemical localization of Inhibin and Activin-like proteins in the brain, pituitary gland and the ovary of *Liza ramada* (Risso). Gen. Comp. Endocrinol., 132: 434–443.

Mousa, S.A. and M.A. Mousa (1999). Immunocytochemical and histochemical studies on the hypophyseal-gonadal system in the freshwater Nile Tilapia, *Oreochromis niloticus* (L.), during sexual maturation and spawning in different habitats. J. Exp. Zool., 284 : 343–354.

Render, J.H., B.A. Thompson and R.L. Allen (1995). Reproductive development of striped mullet in Louisiana estuarine waters with notes on the applicability of reproductive assessment methods for isochronal species. Trans. Amer. Fish. Soc., 124 (1): 26-36.

Sabet, S.S., I.M. Reza, A.F. Bagher and G. Saeed (2009). Study on sexual maturity and levels of gonad steroid hormones in female *kutum Rutilus frisii kutum* (Kamenskii, 1901) during spawning season from river Sefid-Rood of the Southern Caspian Sea. J. Cell Anim. Biol., 3:208–215.

Schulz, R.W., L.R. de França, J-J. Lareyre, F. LeGac, H. Chiarini-Garcia, R.H. Nobrega and T. Miura (2010). Spermatogenesis in fish. Gen. and Comp. Endocrinol., 165:390-411.

SPSS (2012). SPSS Users guide statistics version 19. Copyright IBM, SPSS inc. USA.

Tang, Y.A. (1964). Induced spawning of striped mullet by hormone injection. Japanese J. Ichthyol., 12 (1-2): 23-28.

Wendelaar, B.S.E. (1997). The stress response in fish. Physiol. Rev., 77: 591–625.

Zohar, Y. and C.C. Mylonas (2001). Endocrine manipulations of spawning in cultured fish: From hormones to genes. Aquac., 197:99-136.

استحداث التبويض في أسماك الطوبارة *Liza Ramada* خارج موسم التفريخ الطبيعى

محمد عبد الهادى عبدالمنعم نايل – عبد الحليم على الدروانى - عبد المجيد السيد نصر – خالد محمد المراكبى

قسم الإنتاج الحيواني - كلية الزراعة - جامعة الزقازيق- مصر

صممت الدراسة لتحديد الاحتياجات الهرمونية المناسبة لإحداث التبويض فى أسماك الطوبارة المرباة فى المياه الشَروب داخل الأحواض الترابية، تم تجميع ثمانية مجموعات (٦ أناث / مجموعة) ووضعت داخل أحواض استزراع ترابية، لم يتم حقن المجموعة الأولى (مجموعة المقارنة)، وفى ابريل عام ٢٠١٤ حقنت السبعة مجموعات الأخرى فى العضل على النحو التالي: المجموعة الثانية تم حقنها بـ٤٥٠٠ وحدة دولية / كجم من وزن الأسماك بهرمون النساء الحامل (hCG)، المجموعة الثالثة تم حقنها بـ ٣ ملجم من هرمون الميلاتونين (ML)/ سمكة، المجموعة الرابعة تم حقنها بمستخلص من ٣ غدد نخامية من أسماك المبروك (cPE) / سمكة، المجموعة الخامسة تم حقنها بـ مزيج من ٤٥٠٠ وحدة دولية لهرمون النساء الحامل ومستخلص من ٣ غدد نخامية من أسماك المبروك/سمكة، المجموعة السادسة تم حقنها بمزيج من ٤٥٠٠ وحدة دولية لهرمون النساء الحامل و٣ ملجم من هرمون الميلاتونين/سمكة، المجموعة السابعة تم حقنها بمزيج من ٤٥٠٠ وحدة دولية لهرمون النساء الحامل و مستخلص من ٣ غدد كظرية/سمكة، المجموعة الثامنة تم حقنها بمزيج من مستخلص ٣ غدد نخامية من أسماك المبروك و٣ ملجم من هرمون الميلاتونين/سمكة، وقد أشارت النتائج إلى أن مستوى هرمون استراديول- ١٧ بيتا في مصل الدم قد ارتفع معنوياً فى المجموعة الثانية، وكذلك فإن دليل المناسل الجسمى قد ارتفع معنويا ($P < 0.05$) مع المجموعة الثانية، وقد وجدت زيادة معنوية لكل من تركيز البروجسترون والنسبة بين البروجسترون إلى الاستراديول -١٧بيتا فى السيرم مع المجموعة الخامسة عن باقى المجموعات، وقد فاقت المجموعة الخامسة المجموعات الأخرى من حيث النضج والتبويض، وقد حدث فيها تبويض للحويصلات الناضجة وأخرى في مراحل النضج المختلفة بأسماك الطوبارة خارج موسم التكاثر، وقد خلص البحث إلى أن الحقن بمزيج من ٤٥٠٠ وحدة دولية لهرمون النساء الحامل ومستخلص ٣ غدد نخامية من اسماك المبروك نجحت في استحداث نضج البويضات فى اسماك الطوبارة داخل المياه الشَروب تحت ظروف الأسر خارج موسم التكاثر ويوصى باستخدام مستويات أعلى لإحداث التبويض.

المحكمون :

١- أ.د. سعد زكريا محمد أستاذ علوم البحار – كلية العلوم – جامعة قناة السويس.

٢- أ.د. صلاح الدين علي مصيلحي أستاذ باثولوجيا الأسماك وعميد معهد الاستزراع السمكي وتكنولوجيا الأسماك.