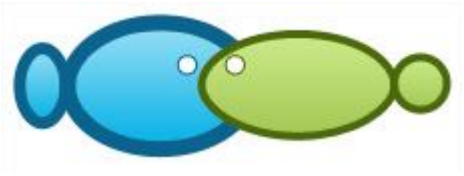

Impact of hormonal manipulation on egg quality of *Diplodus sargus*: comparative ultrastructural changes

Amal F. Fahmy, Zeinab A. El-Greisy, Samia G. Moharram

Aquaculture Division, National Institute of Oceanography & Fisheries (NIOF), Alexandria, Egypt. Corresponding author: A. F. Fahmy, aml.fayez2017@gmail.com

Abstract. This study was carried out to examine the potential of using either gonadotropin-releasing hormone analog (GnRHa) or mixture of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Epigonal) to induce ovulation of white sea bream *Diplodus sargus* during the pre-spawning season. In addition, the effects of different hormonal injections on egg quality by using scanning and transmission microscopy were described. It investigates the differences in the oocyte size and ultrastructural changes of the oocytes surface and zona radiata (ZR) thickness between the two different hormonal therapies. Captured mature females with oocytes diameters > 680 µm were injected twice with two different hormonal protocols. The first group of females was injected intramuscularly with two doses of GnRHa (0.05 µg kg^{-1}) at 24 hrs interval; the second group were injected intramuscularly with two doses of Epigonal (75 IU) at 24 hrs interval. The results revealed that better induction of the egg ovulation with a significant positive correlation between zona radiata (ZR) thickness and oocyte diameters after 12 hrs from the first injection of Epigonal. On the other hand, after the same time of the first injection of GnRHa, the females possessed over-ripening eggs and a gradual onset of residual yolky oocytes. Interestingly, after the second injection of both protocols the oocytes deteriorated, lost their viability and were characterized by stretched egg surfaces with significant differences ($p < 0.05$) between pore diameters and distance between pores. ZR thickness and oocyte diameter showed a reciprocal relationship. This study concluded that the first injection of Epigonal is more effective to induce ovulation of *D. sargus* than GnRHa injection. It is essential to determine the stage of oocyte development, type and doses of hormone injection, the onset of ovulation and the best time for manual stripping to prevent over-ripening eggs in the ovarian cavity that affects egg quality.

Key Words: *Diplodus sargus*, induced ovulation, ultrastructure, over-ripening egg, zona radiata.

Introduction. White sea bream *Diplodus sargus* is one species of highly valued family (Sparidae) in the aquaculture industry of the world. This family is one of the most economically important marine fish families that inhabit the Egyptian coast; it is well represented by a diversity of species and as well both in total landings and high commercial value. Due to the economic importance of this species, it was made the subject of various scientists in different countries (Gonçalves & Erzini 2000; Vigliola & Harmelin 2001; Morato et al 2003; Pajuelo & Lorenzo 2004; Mahmoud et al 2010).

Hormonal manipulation is an important key factor for the sustainability of commercial aquaculture production of wild captive fish. Many different hormonal induction protocols are used efficiently to induce ovulation during artificial propagation of farmed fish species. Hormonal artificial manipulation of the endocrine system acts at different levels in the hypothalamic–pituitary–gonadal axis (Zohar & Mylonas 2001). Gonadotropins secretions have been controlled by gonadotropin-releasing hormone (GnRH) secreted from hypothalamic neurons and is responsible directly of synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary (Gore 2002) and consequently a gonadal secretion of the sex steroids (Poortenaar & Pankhurst 2000). Gonadotropin-releasing hormone analog (GnRHa) injection is used for artificial induced ovulation to mimic the natural secretion of GnRH from the hypothalamus to release LH hormone from the pituitary gland (Pagelson & Zohar 1992). Levavi-Sivan et al (2004) reported that GnRHa injections used to induce ovulation of multiple-batch group-synchronous ovarian development. Hormonal induction

may change the circulating levels of gonadal steroids hormones (Nagahama & Yamashita 2008). They reported the importance of the LH level on the major four sex hormones that regulate the oocyte maturation and egg ovulation in fish. The effective dose of GnRHa was varied between species, and the degree of maturity stages and GnRHa protocol administration (injection, pellet matrix) are important for successful ovulation (Tamaru et al 1988; Park et al 2007; Lim 2016).

Understanding the ova morphology and ultrastructure through scanning and transmission electron microscopy is a potential tool to evaluate the efficacy of hormonal therapy during artificial induction of *D. sargus*. Over-ripening is the process of aging of the oocytes retained in the coelomic cavity (Bromage et al 1994) and it is always the main factor of decreased egg viability (Lahnsteiner 2000). Overripening is characterized by a gradual deterioration of the egg resulting in a semi-transparent yolk in the same ovary. This process of the ovulated eggs in teleost fishes was an important diagnostic key for reduced egg quality (Bromage et al 1992). The accurate time for egg ovulation predicts the best result to obtain the highest eggs quality (Schreck & Moyle 1990). The natural post-ovulation period retained inside the body without over-ripening varies for different fish; over-ripening may occur due to unfavorable environmental conditions (Samarina et al 2015).

The eggs in post-ovulation period were viable, and the duration before over-ripening may extend from hours to days, 10 hrs in *Scophthalmus maximus* (McEvoy 1984) to 9-30 days in rainbow trout *Oncorhynchus mykiss* (Lilley & Rouger 1990). In the period of retention, some batches of eggs for different fish were not viable (overripening) and gradually deteriorated. Aegerter & Jalabert (2004) studied the effects of oocyte ageing in rainbow trout on egg quality problems.

Zona radiata (ZR) and the micropyle of teleost eggs play an essential role in the reproductive success. They are species specific and micropylar ultrastructure is used as an identification tool (Chen et al 2007). Berois et al (2011) found that ZR structures act as bio-monitor for environmental stress. The ultrastructural features of the egg membrane of silver carp Hypophthalmichthys molitrix and micropyle number and size have been used in ichthyology as taxonomic tool (Esmaeili & Johal 2005). Rizzo et al (2003) studies the egg surface, micropyle opening during over-ripening when egg losses viability. Li et al (2000) observed the pores on the ZR of marine fish eggs in three genera in Perciformes. They revealed that the pore diameter was significantly different only in different genera not in the same genus.

The aim of the present study is to explore the best protocol; gonadotropin releasing hormone analogue (GnRHa) or mixed FSH and LH hormones (Epigonal), for ovulation induction of white sea bream *D. sargus*. Also, the study describes the effects of different hormonal injections protocols on egg quality using differences in oocyte size, ultrastructural of the oocytes surface and ZR thickness during ovulation induction of *D. sargus*.

Material and Method

Broodstock management and hormonal induction. Matured female fish were captured from the East Coast of the Mediterranean water at Alexandria, Egypt. They were transported to the Research Unit at the NIOF Hatchery; stocked and acclimatized in fiberglass tanks filled with filtered sea water. The experiment was performed during the pre-spawning period in January 2018. Ninety mature females (18.5-20.8 cm, total length; 130-415 g, body weight) were used and divided into three groups. Each group was represented as triplicate tanks. They were fed on fresh trash fish and crab 2% of body weight day^{-1}, 6 days $week^{-1}$. The first group was controlled while other two groups were induced with two different hormonal therapies protocols.

Experimental design

Endocrine induction of final oocyte maturation and ovulation. The present study adopted two hormone therapy protocols; the first one is the injection of the females intramuscularly with two doses of GnRHa 0.05 µg kg^{-1} at 24 hrs interval time (T_1). In the second protocol, the females were injected intramuscularly with two doses of 75 IU of a

mixture of FSH and LH (commercially: Epigonal 75 IU) with a 24 hrs interval time (T_2) (Table 1). Few drops of clove oil were used for anesthesia during the injection of the fish to decrease the effect of stress on endocrine and ovulatory response during handling. Gonadal biopsies were used to determine oocyte development stages and oocyte diameters at the beginning of the experiment and during the artificial induction protocols. The oocytes of five anesthetized females were sampled during the experiment by polyethylene cannula while at the end of the experiment and after 24 hrs of the second injection the small ovarian samples of fresh dead fish *D. sargus* were collected.

Table1

Different doses of hormonal therapies for artificial ovulation induction of *Diplodus sargus*

Treatment	*Hormone*		*Doses*
T_0 (control)	-		-
T_1	GnRHa	1^{st} inj.	0.05 (µg kg^{-1})
		2^{nd} inj.	0.05 (µg kg^{-1})
T_2	Epigonal (mixture of FSH & LH)	1^{st} inj.	75 IU
		2^{nd} inj.	75 IU

In both protocols; after the second injection, fish were anesthetized and sacrificed to obtain the gonads for histological evaluation. Accurate measurements of ZR thickness were made with scanning and transmission electron images during artificially induced ovulation.

Egg fixation for scanning and transmission electron microscopy. The eggs were fixed in 2.5% glutaraldehyde (2.5% glutaraldehyde and 2% paraformaldehyde) solution, both in 0.1 phosphate buffer (pH 7.4, 4°C) for 24 h. Post-fixation was performed in 1.5% osmium tetroxide for 2 hrs at room temperature, and washed four times with phosphate buffer (pH 7.4). Following fixation, the specimens were dehydrated with an ethanol series, the eggs were dried at 30-40°C, glued to stubs coated with 20 nm of gold, and viewed with scanning electron microscopy (SEM). The eggs were also subjected to routine procedures for transmission electron microscopy. In this case, after fixing the tissue pieces were placed in propylene oxide for 60 min, then in pure Epon. The tissues were sectioned at 1 µm and stained with toluidine blue. Sections were examined by light microscope to identify different representative regions to be sectioned. Ultrathin sections were sectioned with a diamond knife and mounted in copper grids, stained with uranyl acetate and lead citrate (Bancroft & Stevens 1982), and examined with a transmission electron microscopy (TEM).

Statistical analyses. Relationships between oocyte diameter and ZR thickness and significant differences were calculated at $p < 0.05$. Moreover, correlation between pore diameters and the intervening distances were studied and compared between the control and test groups. T test measured a significant difference between the means of the pore diameters and distances between pores in both injection protocols. Statistical analyses were performed using Excel statistical Analysis 2010.

Results

Effect of different hormonal induction protocols on artificially induced ovulation. In the control groups all females failed to ovulate while the two hormonal injection protocols groups showed clear differences in the ovulatory female responses during ovulation induction. A first injection of mixed hormone of FSH and LH (Epigonal) was most effective on ovulation induction. In contrast, the majority of the females injected with GnRHa underwent an over-ripening process even after the first injection.

Effect of different hormonal induction protocols on egg surface pores. The pattern of pore distribution in the oocyte surface in control female groups was as appeared as a snaky pored line with irregular arrangements; pore diameter 0.06±0.01 µm and with 0.35±0.05 µm mean distances between pores (Figure 1A). After the 24 hrs from the first injection of the two hormonal protocols, the ovulated egg surfaces were

gradually perforated with a large number of pores. These were scattered uniformly on the egg surface with different distribution patterns regardless of diameters and distances (Figures 1B, 1D). The number of pores in the egg surface area (pore density) of the first protocol (GnRHa) was larger than the number of pores in the same surface area of the second protocol (Epigonal). At the first GnRHa injection, the mean of pore diameter was 0.29±0.14 µm, and characterized with large holes pattern scattering with small distances between pore (Figure 1B). In contrast, after the same time of first Epigonal injection, the pores of egg surface (0.15 ± 0.05 µm in mean diameter) were observed smaller (Figure 1D). Also, pore surface had a crowded distribution with the mean distance between pores 0.24±0.05 µm (Figure 1D). The results showed the significant differences ($p < 0.05$) in pore diameters between the first injections of the two protocols.

After 24 hrs of the 2nd GnRHa injection, the surface of the ZR was smooth with regular arranged small pores (cone shape) with diameter ranging from 0.04 to 0.09 µm with average 0.05±0.05 µm and distributed distance ranging from 0.19 to 0.24 µm with average 0.23±0.1 µm (Figure 1C). On the other hand and after 24 hrs of the 2nd Epigonal injection, the mean diameter of circular pores was 0.07±0.02 µm with uniform distribution; arranged in diamond pattern (Figure 1E). The mean distance between pores was 0.44±0.11 µm. There was a significant difference ($p < 0.05$) between pore diameters between the second injections of both protocols.

Relation between pore diameters, oocyte diameters and distance between pores. The pore diameters decreased linearly in relation to oocyte diameter after second injection. In both protocols, a significant negative correlation between the oocyte diameter and pore diameters was observed during artificially induced ovulation of the two protocols ($R^2 = 0.94$, $p < 0.05$) (Figure 2).

In addition, a linear relationship was found between pore diameters and distance in between pores along the first and second injection of the two protocols. A statistically significant negative correlation ($p < 0.05$) in both protocols between pore diameters and distance between pores was found during artificial ovulation induction (Figure 3).

Ultrastructure of zona radiata. The histological and ultrastructure of ZR before and after artificial hormonal injection of *D. sargus* are shown in Figures 4 and 5. At the onset of the study, females were containing full-grown oocytes with diameter approximately 700-720 µm. The ultrastructure of mature control oocytes of *D. sargus* was characterized with full membranes structure; ZR, granulosa and theca (Figure 4F). The ZR consisted of two layers: a thinner ZR externa and a thicker ZR interna (ZRI). The ZRI had many layers with dark uniform electron-dense with distribution of round pores. After GnRHa injection protocol, the structure of the ZR completely collapsed with irregular break downs and no distinguished layers (Figures 4A, 4B and 4C). The ultrastructural examination of the oocyte walls (ZR) after second artificial induced ovulation in both protocols indicating postovulatory stage, is shown in Figures 4C and 4E.

By SEM photomicrographs observation, the ZR of the matured control oocytes had eight dense layers (Figure 5A). During injection, ZR thickness decreased and condensed which indicated that the oocyte had been stretched (Figures 5B and 5C). *D. sargus* eggs were characteristics with only one micropyle (Figure 6). The micropyle of the over ripening eggs after a second injection of mixed hormones was still remained open even after over-ripening and completely loss the viability.

By TEM examination, ZR was found to be a simple structure and the pore canals lost their path on either side after the first injection of GnRHa (Figure 7A). In contrast, after GnRHa second injection, the ZR of the over ripening eggs was not observed and was completely absent (Figure 7B). The hypertrophied granulosa and follicular epithelial layers with cytoplasm devoid of organelle are shown in Figure 7B. The difference between ZR thicknesses was observed between the first and second injection of Epigonal group amounting to almost being halved after the second injection (Figures 7C and 7D). With oocyte diameters increasing progressively, the ultrastructural changes gradually become obvious in ZR and pore canals become closed at ZR as seen in Figures 7C and 7D.

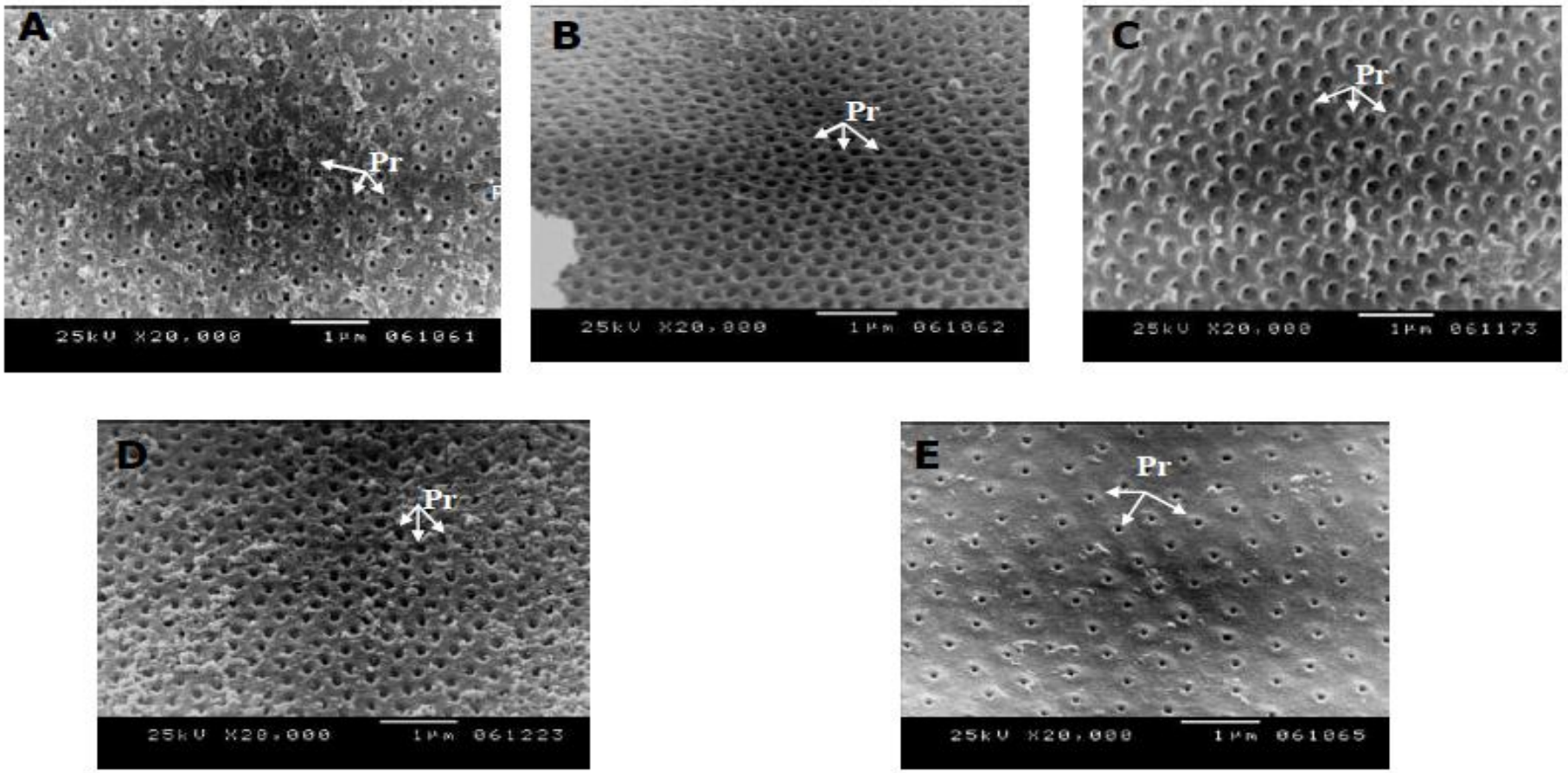

Figure 1. Scanning electron micrographs (SEM) of the oocyte surface at the ovulatory and post-ovulatory oocytes after first and second injection of two hormones in the *Diplodus sargus*, showing (A), Control oocyte showing the differences in the distribution of pores (Pr). (B), After the first injection of GnRHa, (C), After the second injection of GnRHa, (D) After the first injection of Epigonal; (E) After the second injection of Epigonal.

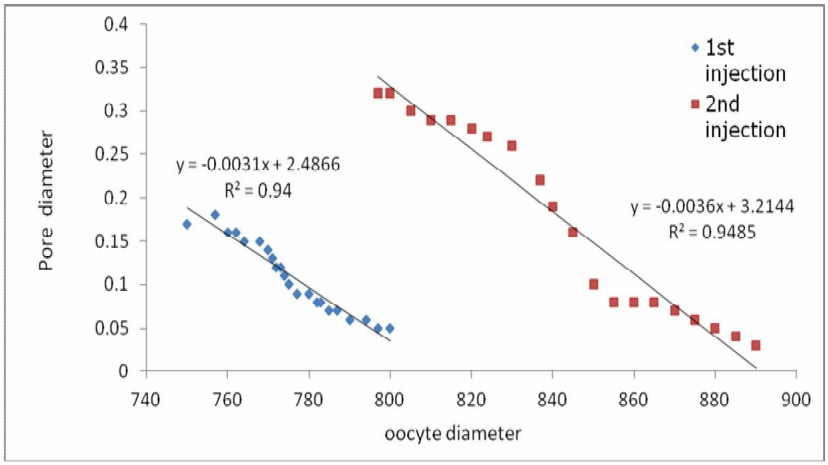

Figure 2. Relationship between oocyte and pore diameters after hormonal injections of the two protocols.

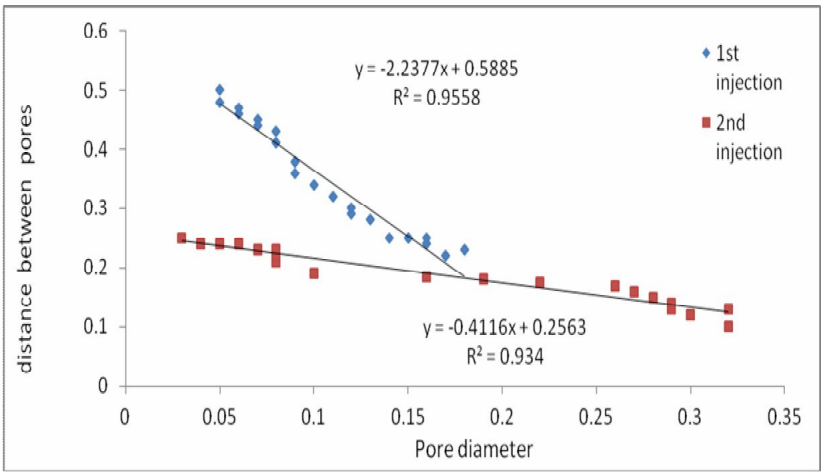

Figure 3. Relationship between pore diameters and distance between pores after hormonal injections of the two protocols.

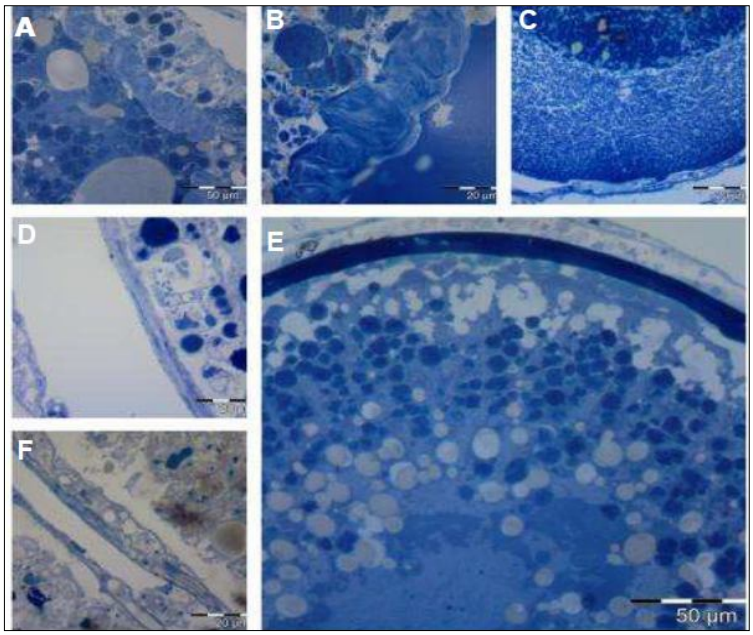

Figure 4. Photomicrograph of semithin section of the oocytes, ovulated and over ripening eggs before and after artificial hormonal injection of *D. sargus*, (A): after first injection of GnRH, (B): after second injection of GnRH, (C): after second injection of GnRH at late overriping stage, (D): after first injection of Epigonal, (E): after second injection of Epigonal, (F): control oocytes.

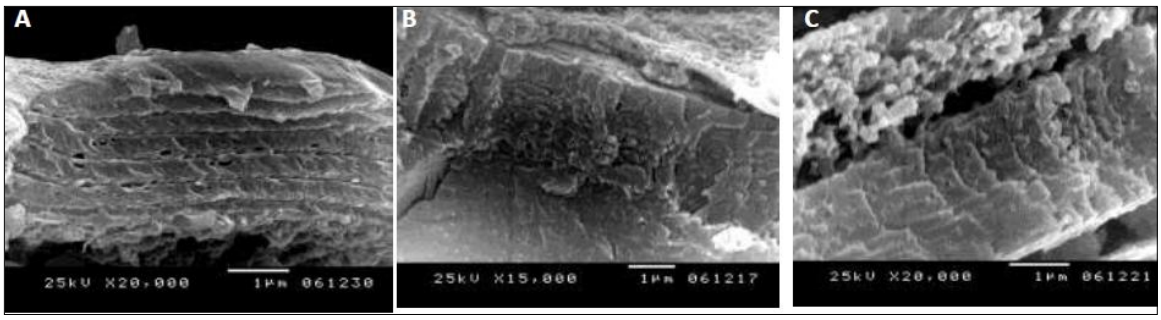

Figure 5. Scanning electron micrographs in (ZR) of control oocytes and ovulated eggs after first injection of two hormones in the *Diplodus sargus* showing the differences measures of zona radiata (ZR) layers. (A): control oocyte, (B): after the first injection of GnRH, (C): after the first injection of Epigonal; the photos showed the compact of ZR corresponding to hormonal injection.

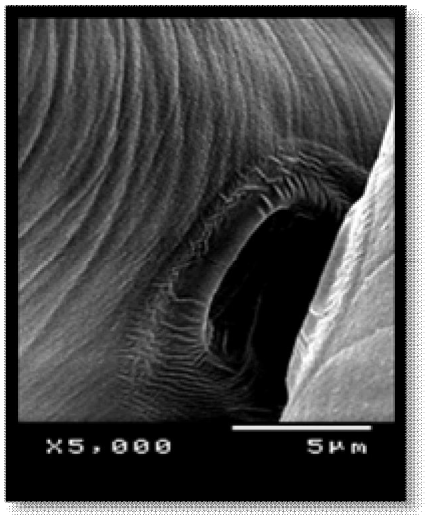

Figure 6. Scanning electron micrographs (SEM) showing micropylar opening of the *Diplodus sargus* over ripening eggs after a second injection of mixed hormones (Epigonal).

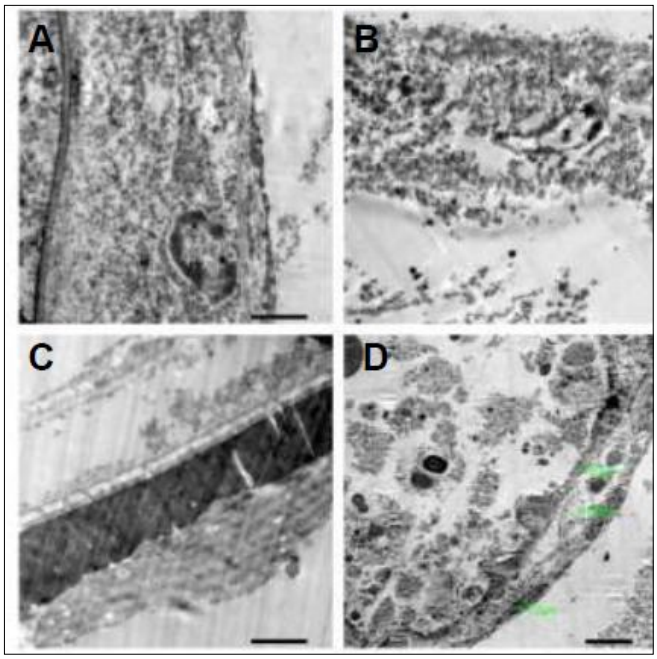

Figure 7. Photomicrograph of TEM in ZR of *Diplodus sargus* oocytes after first and second injection of two hormones. (A): after the first injection GnRH, showing faint radiated ZR; (B): after the second injection of GnRH, showing completely absent ZR; (C): after the first injection of Epigonal, showing closed pore canals; (D): after the second injection of Epigonal, showing deformed structure of ZR.

Relation between zona radiata thickness and oocyte diameters. A linear relationship was found between ZR thickness and oocyte diameters. A statistically significant positive correlation between ZR thickness and oocyte diameters was found when oocyte diameter ranged from 700 to 775 µm ($r = 0.910$, $R^2 = 0.826$, $p < 0.05$) at artificially ovulation of *D. sargus*; whereas, a significant negative correlation was observed when oocyte diameter increased from 780 to 827 µm ($r = -0.949$, $R^2 = 0.899$, $p < 0.05$), during over-ripening stage (Figure 8).

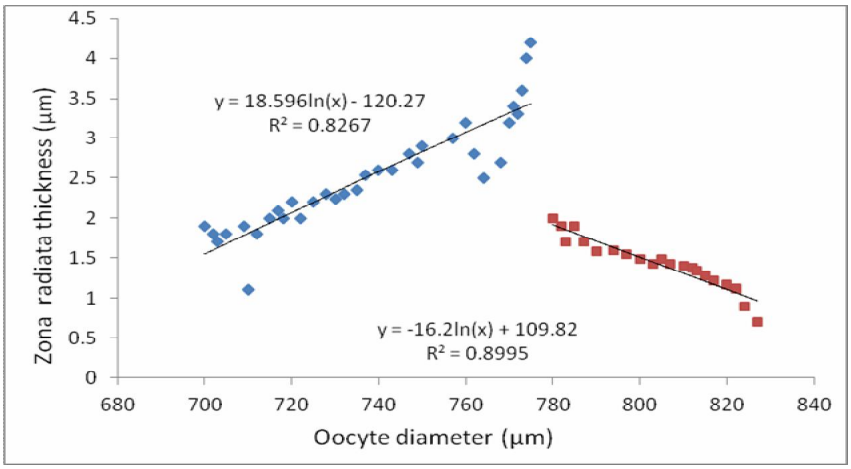

Figure 8. Changes in thickness of chorion in *Diplodus sargus* eggs in relation to oocyte diameters during artificially induced ovulation in both protocols.

Discussion. Reproductive hormones have an impact on the artificial ovulation induction and regulate over-ripening mechanism. Many variables can impact the artificial induction success; type and doses of the stimulating hormones, oocytes size and ZR thickness. The present study demonstrated the effect of two different hormonal induction protocols on *D. sargus* ovulation as evidenced by ultrastructure changes.

The present results showed that the first injection of Epigonal (75 IU) was efficient to induce ovulation in *D. sargus*. Epigonal likes natural pituitary hormones, acted directly on gonads to stimulate the ovulation process. Nagahama & Yamashita (2008) demonstrated the importance of the LH level which influences the synthesis of major four sex hormones that regulate the oocyte maturation and egg ovulation in fish. Epigonal used was sufficient to stimulate ovulation after a few hours of the first injection. The ovulated oocytes retained in the ovarian cavity may depend on the time period which is species-specific. Over-ripening oocyte is one of the most important factors negatively affecting ultrastructure the egg quality. This process may depend on the time of stored these ovulated eggs in the ovarian cavity which consequently affect the quality and obstruct the whole reproductive process. Morphological changes occurring in ovulated eggs retained inside the body ovarian cavity may lead to overripe eggs. This was demonstrated by the oocyte wall changes after the second hormone injection. There was a negative impact on the theca, granuloza and ZR layers. This was observed from twelve hours onwards after the first injection. It means that the manual stripping of the ovulated eggs is may required at an accurate time from the onset of the ovulation. McEvoy (1984) found that using the stripping method of newly ovulated eggs of *Scophthalmus maximus*, the highest fertilization rate and good quality of eggs was obtained. Oocyte ageing may happen according to delayed spawning, delayed egg stripping or environmental stress. Lahnsteiner (2000) studied the effects of doses and time spent in the ovarian cavity on the over-ripening of the ovulated rainbow trout eggs and postulated that all treated females were stripped at a standard 10-11 hrs.

GnRHa injection in the present study may affect the synthesis and release of LH from the pituitary that decrease the LH pituitary content and consequently elevate plasma LH levels. Ismail & Negm (2018) reported that adult female *D. sargus* had maximum size of gonadotroph cells with high granulation (amount of hormone) at the pre-spawning time, which support their endocrine function in reproduction manipulation.

The first dose of GnRHa (0.05 µg kg^{-1}) hormonal injection pushed the oocytes to undergo over-ripening as seen in ultrastructure changes. There are large variation in GnRHa doses as seen in various previous studies which showed that the optimal effective dose of GnRHa is species-specific: 1-5 µg in milkfish (*Chanos chanos*) (Tamaru et al 1988), 70 µg in chum salmon (*Oncorhynchus keta*) (Park et al 2007) and 100 µg of GnRHa pellets in starry flounder (*Platichthys stellatus*) (Lim 2016). Mateos et al (2002) illustrate that GnRHa injection has no effect on FSH hormone synthesis or release but effects the LH synthesis and release from the pituitary.

In the present study, it was found that the ZR thickness of fish increased with increasing oocytes diameter in the ovulatory stage. In contrast, at the onset of post ovulation stage, ZR are stretching and consequently becoming thinner with increasing oocyte diameters. Oocyte quality starts rapidly to decline and lose its viability during over ripening in the ovarian cavity. It was found that ZR thickness decreased even further after 2nd injection of both ovulation induction trials. Earlier, Formation et al (1993) also reported that after 24 hrs of ovulation, the over-ripped eggs were characterized with larger diameters than the newly ovulated egg with a thinner ZR layer. They observed the morphological changes of the outer pore canal during over-ripening in goldfish eggs. In contrast, Rizzo et al (2003) described that no differences were detected between newly ovulated and over-ripened eggs in curimata *Prochilodus marggravii.*

In the present study, it was found that type and dose of hormonal injection had a direct impact on the ultrastructure changes in the ZR thickness and oocytes diameter at ovulation and post ovulation stage. The ZR thickness was significantly thinner after the first dose of GnRHa compared to the first dose of Epigonal group. Following the second injection of both protocols, progressive changes occurred in ZR structure with disruption. Similarly, Craik & Harvey (1984) found that oocyte over ripening strongly correlated with the breakdown of ZR and observed the decrease in the organic molecules in the oocyte membranes. Moreover, Izumi et al (2015) found a positive correlation between oocytes diameters and number of hormonal salmon-pituitary-extract (SPE) injections with ZR thickness of artificially maturing eel.

The work showed a decreased number and diameter of pores on the stretched ZR layer with increase in the distances in between. Therefore, the characteristics of ZR pores may be considered as one of the important guides for reproductive success and egg quality determination.

Interestingly, after the first injection of GnRHa, the number of pores on the egg surface was higher than the Epigonal group. But both groups had less pores after the second dose. This is supported by Groot & Alderdice (1985) who found that the small eggs had a higher number of pores per unit area than larger eggs. Moreover, Li et al (2000) also found significant differences in pore diameter only in the different genera.

A micropyle is another ultrastructure feature in *D. sargus* eggs observed in the study. It functions as a species identification tool and closes off to prevent polyspermy after fertilization (Chen et al 2007). In the present results, it was seen still open in over ripped eggs which has lost its complete viability. Rizzo et al (2003) reported the same results in curimata eggs.

This study is the first description of ZR ultra structure after artificially induced ovulation of *D. sargus* which may help to elucidate the effectiveness of the hormonal induction. It suggests that GnRHa hormone is not effective in inducing ovulation whereas mixed hormone is more effective from the first injection. For high-quality egg production, it is essential to determine the stage of oocyte development, type and doses of hormone injection, the onset of ovulation and the best time for manual stripping to achieve high egg quality and prevent oocyte ageing.

In the present study, oocyte over-ripening is an accurate explanation for the gradual ultrastructural changes occurring in the oocytes of *D. sargus* and loss of oocytes viability after a short time of ovulation obtained from females administered hormonal artificial ovulation induction.

Interestingly, this study shows clear differences in the ovulatory responses between two hormonal injection protocols. A single injection of mixed FSH and LH hormone (Epigonal) was most effective on stimulating ovulation. In contrast, the majority

of the females underwent an over-ripening process from the first injection of GnRHa current dose (0.05 µg kg^{-1}). A lower dose might be effective in achieving better egg quality.

Conclusions. The present study presented the artificial induced ovulation in *D. sargus* by a single dose of mixed FSH and LH hormones (Epigonal) that was sufficient to achieve high egg quality before 12 hrs post injection. The study highlights the importance of harmonizing the stage of oocyte development, type and doses of hormone injection, the onset of ovulation and the best time for manual stripping to achieve high egg quality and prevent oocyte ageing. Further studies are needed to determine the appropriate GnRHa dose to improve the ovulation induction protocols and produce high-quality eggs.

References

Aegerter S., Jalabert B., 2004 Effects of post ovulatory oocyte ageing and temperature on egg quality and on the occurrence of triploid fry in rainbow trout *Oncorhynchus mykiss*. Aquaculture 231(1-4):59-71.

Bancroft J. D., Stevens A., 1982 Theory and practice of histologica techniques. Churchill Livingstone, London, 662 pp.

Berois N., Arezo M. J., Papa N. G., 2011 Gamete interactions in teleost fish: the egg envelope. Basic studies and perspectives as environmental biomonitor. Biological Research 44(2):119-124.

Bromage N., Jones J., Randall C., Thrush M., Davies B., Springate J., 1992 Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture 100(1-3):141-166.

Bromage N., Bruce M., Basavaraja N., Rana K., Shields R., Young C., Dye J., Smith P., Gillespie M., Gamble J., 1994 Egg quality determinants in finfish the role of overripening with special reference to the timing of stripping in the Atlantic halibut *Hippoglossus hippoglossus*. Journal of the World Aquaculture Society 25(1):13-21.

Chen C. H., Wu C. C., Shao K. T., Yang J. S., 2007 Chorion microstructure for identifying five fish eggs of Apogonidae. Journal of Fish Biology 71(3):913-919.

Craik J. C. A., Harvey S. M., 1984 Biochemical changes associated with overripening of the eggs of rainbow trout *Salmo gairdneri* Richardson. Aquaculture 37(4):347-357.

Esmaeili H. R., Johal M. S., 2005 Ultrastructural features of the egg envelope of silver carp, *Hypophthalmichthys molitrix* (Osteichthyes, Cyprinidae). Environmental Biology of Fishes 72:373-377.

Formation M. J., Hori R., Lam T. J., 1993 Overripening of ovulated eggs in goldfish: I. Morphological changes. Aquaculture 114(1-2):155-168.

Gonçalves J. M. S., Erzini K., 2000 The reproductive biology of the two-banded sea bream (*Diplodus vulgaris*) from the southwest coast of Portugal. Journal of Applied Ichthyology 16(3):110-116.

Gore A. C., 2002 GnRH: the master molecule of reproduction. Springer, US, 324 pp.

Groot E. P., Alderdice D. F., 1985 Fine structure of the external egg membrane of five species of Pacific salmon and steelhead trout. Canadian Journal of Zoology 63(3):552-566.

Ismail R. F., Negm R. K., 2018 Histochemical and fine structure studies of pituitary cell type distribution with special emphasis of gonadotrophs during annual reproductive cycle of the adult female white seabream (*Diplodus sargus*). Egyptian Journal of Aquatic Biology and Fisheries 22(5):175-187.

Izumi H., Hagihara S., Kurogi H., et al, 2015 Histological characteristics of the oocyte chorion in wild post-spawning and artificially matured Japanese eels *Anguilla japonica*. Fisheries Science 81(2):321-329.

Lahnsteiner F., 2000 Morphological, physiological and biochemical parameters characterizing the over-ripening of rainbow trout eggs. Fish Physiology and Biochemistry 23:107-118.

Levavi-Sivan B., Vaiman R., Sachs O., Tzchori I., 2004 Spawning induction and hormonal levels during final oocyte maturation in the silver perch (*Bidyanus bidyanus*). Aquaculture 229(1-4):419-431.
Li Y. H., Wu C. C., Yang J. S., 2000 Comparative ultrastructural studies of the zona radiata of marine fish eggs in three genera in Perciformes. Journal of Fish Biology 56(3):615-622.
Lilley N. R., Rouger Y., 1990 Plasma level of gonadotropin and 17α,20β-Dihydroxy-4-pregnen-3-one in relation to spawning behaviour of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Biology 37(5):699-711.
Lim H. K., 2016 Effect of exogenous hormones on ovulation and gonadal steroid plasma levels in starry flounder, *Platichthys stellatus.* Aquaculture International 24:1061-1071.
Mahmoud H. H., Osman A. M., Ezzat A. A., Saleh A. M., 2010 Fisheries biology and management of *Diplodus Sargus Sargus* (Linnaeus, 1758) in Abu Qir Bay, Egypt. Egyptian Journal of Aquatic Research 36(1):123-131.
Mateos J., Mañanós E., Carrillo M., Zanuy S., 2002 Regulation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) gene expression by gonadotropin-releasing hormone (GnRH) and sexual steroids in the Mediterranean Sea bass. Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology 132(1):75-86.
McEvoy L. A., 1984 Ovulatory rhythms and over-ripening of eggs in cultivated turbot, *Scophthalmus maximus* L. Journal of Fish Biology 24(4):437-448.
Morato T., Afonso P., Lourinho P., Nash R. D. M., Santos R. S., 2003 Reproductive biology and recruitment of the sea bream in the Azores. Journal of Fish Biology 63(1):59-72.
Nagahama Y., Yamashita M., 2008 Regulation of oocyte maturation in fish. Development, Growth and Differentiation 50(1):195-219.
Pagelson G., Zohar Y., 1992 Characterization of gonadotropin-releasing hormone binding to pituitary receptors in the gilthead seabream (*Sparus aurata*). Biology of Reproduction 47(6):1004-1008.
Pajuelo J. G., Lorenzo J. M., 2004 Basic characteristics of the population dynamic and state of exploitation of Moroccan white seabream *Diplodus sargus cadenati* (Sparidae) in the Canarian archipelago. Journal of Applied Ichthyology 20(1):15-21.
Park W. D., Lee C. H., Lee C. S., Kim D. J., Kim J. H., Tamaru C. S., Sohn Y. C., 2007 Effects of a gonadotropin-releasing hormone analog combined with pimozide on plasma sex steroid hormones, ovulation and egg quality in freshwater-exposed female chum salmon (*Oncorhynchus keta*). Aquaculture 271(1-4):488-497.
Poortenaar C. W., Pankhurst N. W., 2000 Effect of luteinizing hormone-releasing hormone analogue and human chorionic gonadotropin on ovulation, plasma and ovarian levels of gonadal steroids in greenback flounder *Rhombosolea tapirina*. Journal of World Aquaculture Society 31(2):175-185.
Rizzo E., Godinho H. P., Sato Y., 2003 Short-term storage of oocytes from the neotropical teleost fish *Prochilodus marggravii*. Theriogenology 60(6):1059-1070.
Samarina A. M., Policar T., Lahnsteiner F., 2015 Fish oocyte ageing and its effect on egg quality. Reviews in Fisheries Science and Aquaculture 23(3):302-314.
Schreck C. B., Moyle P. B., 1990 Methods for fish biology. American Fisheries Society, pp. 529-547.
Tamaru C. S., Lee C. S., Kelley C. D., Banno J. E., Ha P. Y., Aida K., Hanyu L., 1988 Characterizing the stage of maturity most receptive to an acute LHRH-analogue therapy for inducing milkfish (*Chanos chanos*) to spawn. Aquaculture 74(1-2):147-163.
Vigliola L., Harmelin M., 2001 Post-settlement ontogeny in three Mediterranean reef fish species of the genus *Diplodus*. Bulletin of Marine Science 68(2):271-286.
Zohar Y., Mylonas C. C., 2001 Endocrine manipulations of spawning in cultured fish: from hormones to genes. Aquaculture 197(1-4):99-136.

Received: 31 May 2020. Accepted: 28 August 2020. Published online: 01 October 2020.
Authors:
Amal F. Fahmy, Laboratory of Fish Reproduction and Spawning, Aquaculture Division, National Institute of Oceanography & Fisheries (NIOF), Kayet-bey Al-Anfoushy, Area Code 21556, Alexandria, Egypt, e-mail: aml.fayez2017@gmail.com
Zeinab A. El-Greisy, Laboratory of Fish Reproduction and Spawning, Aquaculture Division, National Institute of Oceanography & Fisheries (NIOF), Kayet-bey Al-Anfoushy, Area Code 21556, Alexandria, Egypt, e-mail: zeinab_elgreisy@yahoo.com
Samia G. Moharram, Laboratory of Fish Reproduction and Spawning, Aquaculture Division, National Institute of Oceanography & Fisheries (NIOF), Kayet-bey Al-Anfoushy, Area Code 21556, Alexandria, Egypt, e-mail: samiamoharram1@gmail.com

How to cite this article:
Fahmy A. F., El-Greisy Z. A., Moharram S. G., 2020 Impact of hormonal manipulation on egg quality of *Diplodus sargus*: comparative ultrastructural changes. AACL Bioflux 13(5):2664-2675.