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Research Article

# Gonadal Oocyte Development in LHRHa Hormone Treated European Sea Bass (*Dicentrarchus labrax* L., 1758) Broodstock

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**Abstract:** Stages of oocyte maturation in sea bass (*Dicentrarchus labrax*) gonads after LHRHa hormonal treatment were investigated. LHRHa was injected into sea bass broodstock with an oocyte diameter of 527-580 µm. A second injection was given 12 h after the first injection. For each injection a dose of 10 µg/kg was used. At 24 h, oil globules in oocytes were randomly distributed. These oil globules started to fuse and change to a spherical shape from an ellipsoidal shape at 36 and 42 h. Ovulation was observed between 45 and 54 h after the first intramuscular injection. After 54 h from the first injection, oocytes became overripe due to no spawning occurring on time. The overripe rate in gonads was 90% at 60 h.

**Key Words:** Oocyte, hormone, LHRHa, European sea bass

## LHRHa Hormonu Uygulanan Avrupa Levrek (*Dicentrarchus labrax* L., 1758) Anaçlarının Gonadlarındaki Oosit Gelişimi

**Özet:** LHRHa hormonu uygulanmasının ardından levrek (*Dicentrarchus labrax*) balıklarında gonad içi oosit gelişiminin geçirdiği safhalar tespit edilmiştir. LHRHa hormonu gonad içi oosit çapı 527-580 µm arasında olan levrek anaçlarına uygulanmıştır. Hormon uygulaması 12 saat ara ile 10 µg/kg olacak şekilde yapılmıştır. 24. saatte hücre içindeki yağ damlaları dağınık şekildedir. 36-42. saatlerde bu yağ damlalarının birleşmeye başladığı ve elipsoidal yapıdan küresel hale geçtikleri saptanmıştır. İlk enjeksiyondan 45-54 saat sonra ovulasyon izlenmiştir. İlk hormon enjeksiyonundan 54 saat sonra zamanında bırakılmadığı için oositler bozulmaya başladılar. 60. saatte gonadlarda bozulmuş safhadaki yumurtaların oranının % 90 olduğu saptanmıştır.

**Anahtar Sözcükler:** Oosit, hormon, LHRHa, Avrupa deniz levreği

## Introduction

European sea bass (*Dicentrarchus labrax*) is the most important teleost fish for aquaculture in the Mediterranean area. This species spawns spontaneously in captivity, but spawning in commercial hatcheries is still inconsistent and unreliable (1). Spawning is especially problematic when environmental conditions differ from natural ones, e.g., high temperature and shifted photoperiod (2,3).

In fish culture, spawning can be induced by environmental manipulation and/or exogenous hormone administration (4). In captivity, induction of spawning by hormonal treatment is necessary for species that do not spawn spontaneously. However, this technique might also be very useful in synchronising spawning in those species which spawn normally in culture conditions. Synthetic agonists of the gonadotropin-releasing hormone GnRHa have been used extensively in the last 2 decades to induce the necessary synthesis release of LH from the pituitary, resulting in final oocyte maturation (FOM), ovulation and spawning (5-9). The use of GnRHa-delivery systems in female broodstock can also improve the quantity and quality of the eggs produced when compared with those of spontaneously spawning fish (10,11) and fish treated with GnRHa injections (12).

Hormonal treatment is important due to synchronic spawning and oocyte development in gonads and also in determining time of spawning. There are some studies on the characteristics of eggs after hormonal treatment. The purpose of this study was to determine stages of oocytes at the vitellogenesis stage after hormonal treatment and stages of overripe eggs after ovulation by monitoring techniques.

## Materials and Methods

Adult sea bass females and males, reared under natural photoperiods and temperatures in the Teknomar Sea Fish Broodstock Centre, Izmir, Turkey, were distributed as 4 females (4.2 ± 0.12 kg mean weight) in vitellogenesis and 8 spermiating males (2.9 ± 0.09 kg mean weight). The experiment was carried out in mid-December. Each group of fish was kept in separate well-aerated lightproof 5000-l capacity tanks with an open circuit of sea water salinities of 37‰ and fish were maintained in natural conditions of a photoperiod of 9 h light:15 h dark and a temperature of 15 ºC throughout the experiment. Oxygen saturation was over 85% and pH was around 7.65. Ammonia and nitrite components were always less than 0.012 mg/l. No food was provided during the experiment.

Fish were marked on the dorsal fin and named L-0, L-1, L-2, L-3 and Control (C). LHRHa (D-Ala, Pro - NEt–LHRHa) was used in 10 µg/kg doses 12 h apart for female breeders and the amount of LHRHa was obtained by diluting it with physiological saline. No hormonal administration was applied for control breeders (C). Fish were anaesthetised with phenoxyethanol (150 ml/$m^3$) before hormonal treatment. LHRHa treatments were injected into the second dorsal sinus by making a 45º angle and a second injection was applied 12 h after the first one. After the first injection, oocyte maturation was observed at 12 h intervals for the first 36 h and at 6 h intervals between 36 and 66 h, and the study was terminated with oocyte ovulation. In addition, oocyte maturation stages and diameters were estimated. The first hormonal administration was described as 0 h.

Oocyte samples of a minimum of 100 oocytes each were taken from the gonads using catheter tubing with an inner diameter 1.87 mm, and then they were examined under a light microscope. Images of the different oocyte stages were taken using a Nikon Coolpix 5000 digital camera. Data are presented as mean ± S.E. Differences between oocyte diameters in the experimental and control groups in were determined by one-way analysis of variance (ANOVA, $P < 0.05$).

## Results

No differences were observed in whole oocytes in L-0, L-1, L-2 and L-3 breeders before the first injection and at early stage vitellogenesis (immature) Figure 1 a. However, at the vitellogenesis stage we observed approximately 10% of oocytes from L-3.

However, 12 h later oocytes in L-3 gonads were similar to the formations which were observed at 0 h. No significant difference was determined between the oocytes of L-0, L-1 and L-2 gonads after 12 h ($P > 0.05$).

Undifferentiated oocytes with more oil globules were observed 24 h after from the first injection. Additionally, in L-2 gonads, either mostly infused oil globule or partly fused oil globule undifferentiated oocytes were determined. Oil globules accumulated and decreased quantitatively, and immature oocytes were observed in L-3. The percentage of undifferentiated oocytes was 30%. The accumulation of oil globules is shown in Figure 1 b.

Although oil globules started to fuse at 36 h, there were many immature oocytes in L-0 and L-1. This was more homogeneous in L-2 and oil globules were ellipsoidal. Oil globules were fused and enlarged; however, oocytes became semi-transparent and the transparency of oil globules increased (Figure 1 c).

At 42 h, oil globules started to change to spherical from ellipsoidal in L-0, L-1 and L-2, and oocytes were not transparent. Oil globules became completely spherical and oocytes were transparent in L-3. In addition, the number of oil globules decreased and changed between 1 - 4. Almost no deformity in oocytes was observed.

After 45 h, recannulation was maintained in L-3 breeders due to development of fish and larger initial diameters of oocytes. In addition, there were many overripe oocytes in the anterior and posterior parts of the gonads.

Oocytes in L-0, L-1 and L-2 gonads became more transparent and spherical at 48 h, but a few immature oocytes were observed in L-0 and L-1 gonads. It was remarkable that oil globules in L-2 gonads were in more pieces, little and scattered. Additionally, oocytes in L-3 gonads were overripe. Findings at 54 h were parallel to those at 48 h.

At 60 h, either immature and mature or abnormal oocytes were observed in L-0 and L-1 gonads. The formation of too many oil globules continued in L-2 oocytes and all the oocytes in L-3 gonads were overripe.

The ovulation stage was determined between 45 and 54 h in L-0, L-1 and L-2, but occurred between 42 and 45 h in L-3 gonads, and after these times oocytes became

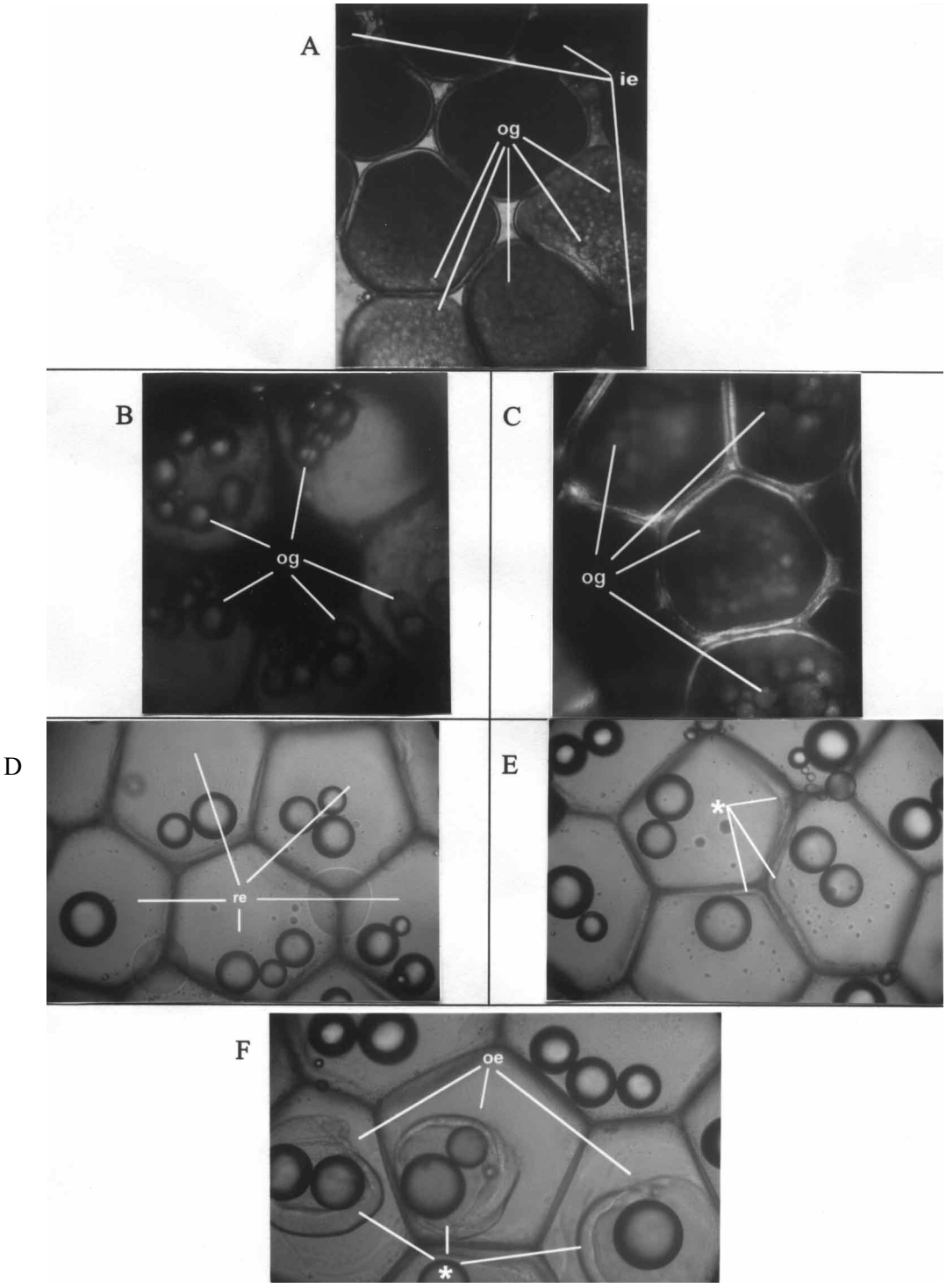


Figure 1. a) Oocytes before hormone treatments and in the control group during the experiment (ie: immature egg, og: oil globule). b) The accumulation of oil globules. c) Oocytes became semi-transparent and transparency of oil globules. d) Oocytes in ovulation stage (re: ripe eggs). e) Breakdown at the inner surface of chorion (*: breakdown). f) Dead eggs (oe: overripe oocytes).

overripe. Oocytes in the ovulation stage, breakdown at the inner surface of the chorion and overripe oocytes are shown in Figures 1 d – f, respectively.

Initial diameters of oocytes were 527 ± 11.56 µm for L-0, 539 ± 8.64 µm for L-1, 567 ± 13.87 µm for L-2, 580 ± 18.56 µm for L-3 and 535 ± 14.42 µm for control fish, and no significant differences were calculated among the diameters ($P > 0.05$). At the end of the experiment, mean diameters of oocytes were 1012 ± 28.51 µm, 1035 ± 19.46 µm, 998 ± 20.32 µm, 1045 ± 17.51 and 1037±26.93 µm for L-0, L-1, L-2 and L-3, respectively ($P > 0.05$) but were unchanged in control fish (538 ± 12.34 µm) ($P > 0.05$). There were significant differences between the groups and the control oocyte diameters ($P < 0.05$).

## Discussion

Gonadal oocyte development in sea bass during the ovulation period was determined by using LHRHa. LHRHa was applied to the sea bass breeders and the broodfish oocytes diameters varied between 527 ± 11.56 µm and 580 ± 18.56 µm. The diameters of the oocytes in this experiment were suitable for inducing ovulation and synchronic spawning. When GnRHa was used on female sea bass, 100% ovulation was observed in oocytes larger than 600 µm (end of vitellogenesis) (13,14). In spite of this range in our study, ovulation was observed in most parts of the gonads. It might be thought that this difference resulted from oocyte diameter, broodstock spawning performance, and the quantity of hormone used during treatment. It was reported that the hormonal type and dose used in this study had been applied by several authors without any problem in this species.

In previous studies, it was recorded that 2 injections caused oocyte maturation (13) and spawning (15), and these usually occurred 3 days after treatment (2) in sea bass. In this study, we observed that between 48 and 54 h after LHRHa treatment, oocytes started to be released synchronously by broodfish, and breeders in this experiment achieved ovulation earlier than in previous records (13,15). This is explained by the fact that differences in natural conditions such as temperature, food, geographical location and broodstock management operations such as salinity, temperature, and nutrition protocols in captivity could affect the broodstock's physiological behaviour.

Egg quantity, rates of viable egg and larval deformity were calculated in order to determine the hormonal treatment effects. In addition, stress is one of the main factors in spawning, and in the current study no spawning was observed due to manipulation.

In conclusion, it is important that successful broodstock management produce high quality eggs, and, in turn, quality eggs are a guarantee that the maximum number of larvae and fry will survive. As in striped bass (16), it is possible that ovulated but unspawned fish due to biotical and abiotical conditions could spawn and be fertilised outside. The exact time of ovulation and description of developmental stages of oocytes should be determined by further studies. This could also be useful for ovulated but unspawned fish in order to prevent gonadal quality damage due to negative effects of reabsorption; however, it is clear that requirement of quantity egg was provided synchronously by breeders.

In addition to this, the effects of LHRHa on egg quality, egg viability, and larval deformation are other important issues that should be examined in the future.

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