Hormonal Induced Spawning of Marine Sea Bass *Dicentrarchus labrax* L.

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Abstract: Sea bass brood stocks were caught with average body weight of 2000.0 ± 200 g for males and 3000.0 ± 400 g for females, from fish farms at Damietta governorate. Brood stocks transported to a private fish farm, situated in Al-Qantara Gharb, Ismailia governorate, Egypt. A total of 72 brood stocks were divided into four groups with 3 replicates for each group to evaluate the egg production under the effect of different hormones. Each group consists of 4 males and 2 females otherwise the sex ratio was two males to one female. Brood stocks were injected when the water temperature reached 14.8°C during January. The four groups (males and females) were injected as follows; T_1: The control group, brood stocks were injected with physiological saline (0.9% NaCl), T_2: injection with 25 ml hCG kg^{-1} body weight**.** T_3: injection with 50 ml LH-RH kg^{-1} body weight. T_4: injection with two doses: the first dose was injected at 10 am with 25 ml LH-RH kg^{-1} body weight and the second dose was injected with 12.5 ml hCG kg^{-1} body weight after 24 hrs from the first dose. Fertilization percentage was recorded in group which injected twice with LHRH and hCG (T_4). The injection of LHRH repaired the endocrine disruption that results in the failure of captive fish to undergo FOM, ovulation and spawning, but hCG could not effect on oocyte development before releasing eggs, but could effect on latency period and time period of releasing eggs. Therefore, this is an important step towards the domestication and culture of sea bass (*Dicentrarchus labrax*), which considered a good aquaculture potential species.

Keywords: Induced spawning, *Dicentrarchus labrax*, hCG, LHRH

INTRODUCTION

Sea bass (*Dicentrarchus labrax L.*) is a marine teleost fish of great economic value in Europe and the Mediterranean area (Firat *et al.,* 2005). In the wild, sea bass spawns during winter months (from December to March) at low temperatures (12 – 14˚c) and at short or increasing day length (FAO, 1999). It has no morphologically distinct sex chromosomes, differentiation of the gonads not reaching completion until almost the end of the first year of life. Genetic and environmental factors influence the age of sexual maturation. They present a high fecundity (on average 200,000 eggs/kg of female), start to reproduce over 2kg and can reach 6 to 7 years in the wild (Froese and Pauly, 2013). Photo period is one of the most important cues in triggering puberty and reproduction in this species. Females naturally reach puberty a year later than males and are generally larger in size.

Almost all fish reared in captivity exhibit some form of reproductive dysfunction. In females, there is often failure to undergo final oocyte maturation, ovulation and spawning (Zohar, 1988; Zohar, 1989a and b; Peter *et al.,* 1993), while in males milt production may be reduced and low quality (Billard, 1989; Cosson, *et al.,* 2008). These dysfunctions are due to the fact that, fish in captivity do not experience the conditions of the spawning grounds, and as a result there is a failure of the pituitary to release the maturational gonadotropin, luteinizing hormone (LH). Induced spawning of captive fish may be approached in two ways, hormonal and environmental treatments. In some species hormonal treatments are the only means of controlling reproduction reliably.

In captivity, Hormonal manipulations are used only for non-responsive fish reared under artificial photo thermal conditions such as sea bass (Barbaro *et al.,* 1997). Induction of spawning using hormones provides a direct control over the final stages of the reproductive cycle in teleosts.

This study was conducted to investigate the effect of injecting fish with Luteinizing hormone releasing hormone and human chorionic gonadotropin hormone as well as the effect of different doses of the hormone on the induced spawning and fertilization percentage of sea bass.

MATERIALS AND METHODS

1- Brood stock selection

Sea bass brood stocks were caught with average body weight of 2000.0 ± 200 g for males and 3000.0 ± 400 g for females, from fish farms at Damietta governorate. Brood stocks transported to a private fish farm, situated in Al-Qantara Gharb, Ismailia governorate, Egypt.

Brood stocks were acclimatized to laboratory conditions for about one week prior to spawning. Males were anaesthetized with MS222 (200 mg/L) to be identified by the presence of freely expressible milt under abdominal pressure, while the state of oocyte development of females was confirmed by ovarian biopsy. A small sample of oocyte was taken from females by inserting a catheter (canola) into the genital pore and examined.

2- Nutrition:

The brood stock were manually fed twice daily to satiation throughout the spawning season with fresh pieces of (squad, mackerel, sardine, shrimp and small fish).

3- Water Physico-chemical parameters:

Brood stock reared in sea water, which filtered with sandy filters. The water parameters were controlled as following: temperature of 18 – 14˚C by thermometer (model, HI 146-99) and salinity of 35 ppt by Refractometer, pH 7-8 by pH meter (model, pH222). Dissolved oxygen level was 6 to 8 mg/l by using air blower, provided through diffuser stones and water flow percentage of one liter per minute using inlet-outlet system.

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Fish were kept in outdoor concrete tanks with maximum capacity of 10 m^3 of sea water, under natural photoperiod.

4. Experimental design and hormones:

Fish were injected intramuscularly above the lateral line, just below the dorsal fin. The injected area was rubbed with a finger in order to distribute the hormone evenly throughout the muscle and to prevent a backflow of the hormone.

Two hormones were examined to induce spawning, the first was human chorionic gonadotropin hormone (hCG, Argent labs., Redmond WA Washington, United States 98052) and the second was Luteinizing hormone-releasing hormone (LHRH, Gonadotrophin-releasing hormone, Argent labs Redmond WA Washington, United States).

hCG and LHRH dilutions preparation by dissolving the contents of the hormone package by 10 cm of 0.9 saline solution (0.9% NaCl).

A total of 72 brood stocks were divided into four groups with 3 replicates for each group to evaluate the egg production under the effect of different hormones.

Each group consists of 4 males and 2 females and the sex ratio was 2:1.

Brood stocks were injected when the water temperature reached 14.8 °C during January.

The four groups (males and females) were injected as following;

Treatment (1): The control group, brood stocks were injected with physiological saline (0.9% NaCl).

Treatment (2): Brood stocks were injected with 25 ml hCG kg^{-1} body weight (2,500 IU hCG/kg body weight) (Personal communication).

Treatment (3): Brood stocks were injected with 50 ml LH-RH kg^{-1} body weight (50 µg LHRH/kg body weight) (Personal communication).

Treatment (4): Brood stocks were injected with two doses: the first dose was injected at 10 am with 25 ml LH-RH kg^{-1} body weight (25 µg LHRH/kg body weight) and the second dose was injected with 12.5 ml hCG kg^{-1} body weight (1250 IU hCG/kg body weight) after 24 hrs from the first dose (Personal communication).

Follow-up the launch of the latency period, time period of releasing eggs and the proportion of fertilization. The induced fish were observed to spawn 48-52 hrs after hCG hormonal injection and 3 days after LHRH and LHRH+hCG hormonal injection. The buoyant eggs were directed towards the overflow opening by the gentle inlet water current. Pelagic eggs were collected four times a day in fine meshed 300 µ collection buckets placed at the overflow waters from the spawning tanks. Then, the eggs were washed thoroughly with new seawater and placed in a measuring cylinder for separation of good buoyant fertilized eggs from sinking bad eggs as well as for volumetric estimates. Fertilized eggs, unfertilized eggs, fertilization percentage, latency period, time of releasing eggs and mortality were calculated.

Fertilization percentage % = (Number of fertilized eggs/Total number of counted eggs) x 100

Latency period: it is the time between injection and releasing eggs.

Time period of releasing eggs: The time between the start and the end of releasing eggs.

RESULTS

1) Releasing eggs of sea bass:

Fish were injected when the water temperature reached to 14.8°C (as an average) during January in a natural photoperiod with two hormones hCG (Human Chorionic Gonadotropin Hormone), LHRH (Luteinizing hormone releasing hormone) and their mixed dosage into three treatments. The highest value of fertilized eggs was recorded in T_3 group, which injected with LHRH and the lowest value was recorded in the fish group which injected with hCG T_2 (Table 1 and Fig. 1)

The highest value of non-fertilized eggs was recorded in T_2 group, which injected with 25 ml hCG kg^{-1} body weight and the medium value recorded in the group injected with 50 ml LHRH kg^{-1} body weight (T_3) otherwise the lowest value was recorded in group injected with 25 ml LHRH kg^{-1}+ 12.5 ml hCG kg^{-1} body weight (T_4). The best value of fertilization percentage in observed fish group injected with LHRH+hCG (T_4) and the medium value was recorded in T_3 group which injected with LHRH (Table 1 and Fig. 2). The latency period was longer in both group T_3 and T_4 (Table 1 and Fig. 3). The time of releasing eggs (Table 1 and Fig. 3) was longer in the fish group injected with LHRH (T_3) and the medium releasing time was observed in T_2 group which injected with hCG, while the short releasing time was recorded in fish group injected with LHRH+hCG (T_4).

Table (1): The effect of hCG, LHRH and (hCG + LHRH) hormones on fertilized eggs, fertilization percentage, latency period and time period of releasing eggs of sea bass, *Dicentrarchus labrax*.

	Control T_1	hCG T_2	LHRH T_3	LHRH+hCG T_4
Number of fertilized eggs ($x10^6$)	**0.00**	**3.53 ^{c} ± 0.064**	**4.72 ^{a} ± 0.148**	**3.98 ^{b} ± 0.10**
Number of non-fertilized eggs ($x10^6$)	**0.00**	**0.85 ^{a} ± 0.129**	**0.52 ^{b} ± 0.06**	**0.34 ^{b} ± 0.022**
Fertilization rate (%)	**0.00**	**75.63 ^{b} ± 3.78**	**89.27 ^{a} ± 1.37**	**91.5 ^{a} ± 0.5**
Latency period (h)	**0.00**	**51.33 ^{b} ± 1.76**	**96 ^{a} ± 0.00**	**96 ^{a} ± 0.00**
Time period of releasing eggs (h)	**0.00**	**68 ^{b} ± 4**	**152 ^{a} ± 8**	**48 ^{c} ± 0.00**

Means in each row with different superscripts letters are significantly different ($P<0.05$).

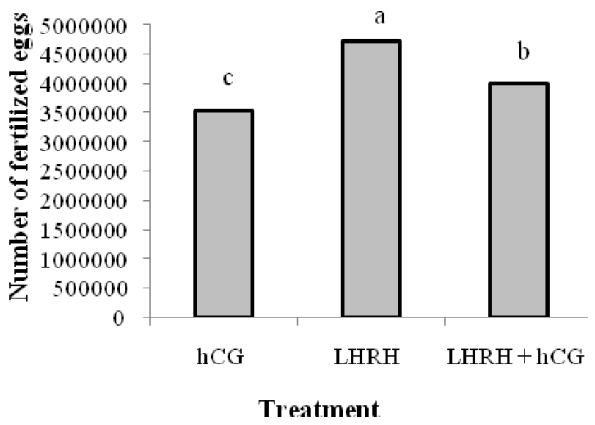

Fig. (1): The effect of hCG, LHRH and (hCG + LHRH) hormones on fertilized eggs of sea bass (*D. labrax*)

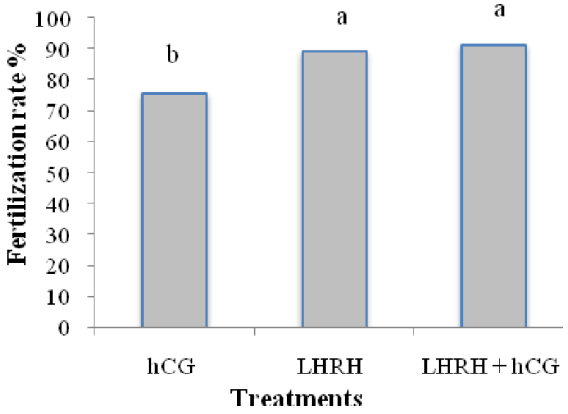

Fig. (2): The effect of hCG, LHRH and (hCG+LHRH) hormones on fertilization percentage of sea bass (*D. labrax*)

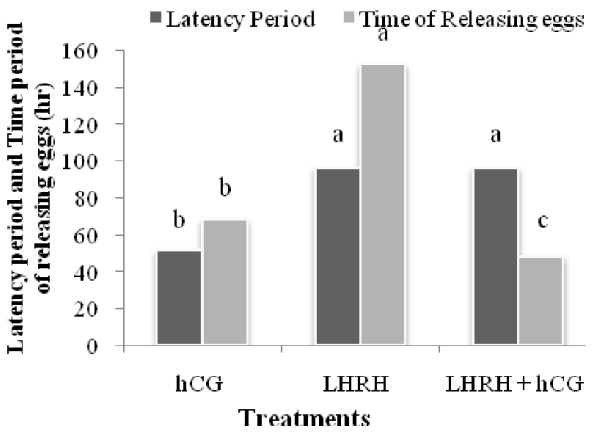

Fig. (3): The effect of hCG, LHRH and (hCG+LHRH) hormones on latency period and time period of releasing eggs of sea bass (*D. labrax*)

2) Eggs production during the experimental periods:

Eggs production during the experimental periods was recorded and the fertilization percentage (%) in each period to determine the best time to collecting good eggs.

Figures 4 and 5 showed the highest and lowest values of eggs production and fertilization percentage during the experimental periods.

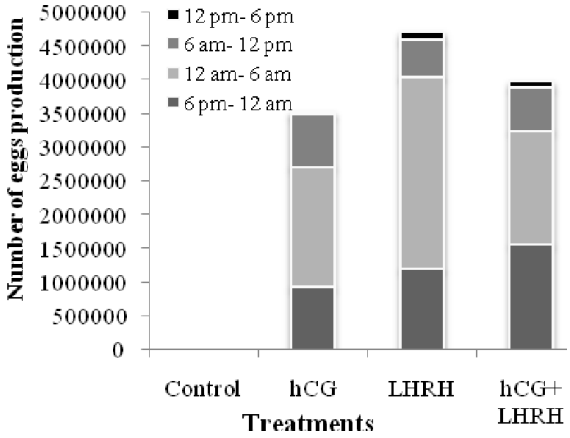

Fig. (4): Egg production during the experimental periods of sea bass (*D. labrax*)

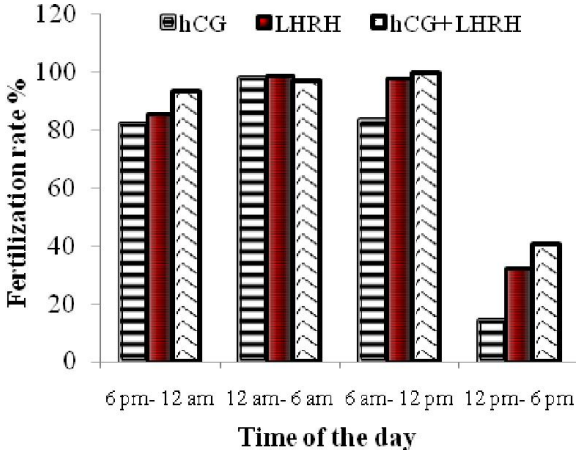

Fig (5): Fertilization percentage (%) of sea bass (*D. labrax*) during the experimental period

DISCUSSION

Many fish spawn in environments that are nearly impossible to simulate in a hatchery. Hormone-induced spawning is the only reliable method to induce reproduction in these fishes. In this study, results clearly show that the fertilized and non-fertilized eggs, fertilization percentage, latency period and time period of releasing eggs can be very different due to use different hormones. Using of LHRH and hCG induced the maturation processes and egg release. Fish were injected when the water temperature reached 14.8°C and in each group there are one female in final oocyte maturation (FOM) and one female was in the stage before FOM.

The female of sea bass in FOM, in the stage before and males were injected once with 2,500 IU hCG/kg of body weight (25 ml/kg). Spawning occurs between 24-72 hours after a single injection. The poorest results were recorded in this group of fertilized and non-fertilized eggs and fertilization percentage due to hCG had no effect on oocyte development before releasing eggs and the advantage of hCG is that it acts directly at the level of the gonad and does not require the existence of LH stores or activation of the pituitary gonadotropes which agreement with Hodson and Sullivan (1993) and the injected gonadotropin mimics the natural GtH

produced by the fish's pituitary. Just as is the case with pituitary extracts, purified hormones such as HCG bypass the brain-pituitary link, acting directly on the ovaries and testes, but the fastest results of latency period and time period of releasing eggs were recorded in the group which injected with hCG.

For sea bass, it must be stressed that the injection of LHRH, on a large scale during normal or shifted spawning seasons gives a good a performance as natural spawning similar to those obtained by LHRH, on an experimental scale (Barnabé and Barnabé-Quet, 1985 and Barbaro *et al.,* 2002). LHRH have potent stimulator effects on ovulation and spermiation in fishes. Therefore, only one or two small doses are needed to induce spawning. LHRH stimulates the fish's own pituitary to produce and release the GtH necessary for spawning which agreement with Rottmann *et al.* (1991). Due to LHRH acts at a higher level of the hypothalamus–pituitary–gonad axis, LHRH can provide a more balanced stimulation of reproductive events and, presumably, a better integration of these events with other physiological functions, by directly or indirectly affecting the release of other hormones necessary for successful FOM, spermiation and spawning, which nearly agree with Zohar and Mylonas (2001) and Ergun (2002).

In the present study, results revealed that oocytes started to be released synchronously by brood stock, and breeders between 3 and 4 days after LHRH treatment (T_3), and achieved ovulation such as the previous records of (Alvariano *et al.,* 1992). This is explained by the fact that differences in natural conditions such as temperature, feed, geographical location and brood stock management operations like salinity, temperature, and nutrition protocols in captivity could affect the brood stock physiological behavior. The group of T_3 gives good results of fertilization percentage, but it takes a longer time more than hCG (T_2) in latency period and time period of releasing eggs.

In this study, the best results of fertilization percentage were recorded in the group injected twice with LHRH and hCG (T_4). The injection of LHRH repaired the endocrine disruption that resulted in the failure of captive fish to undergo FOM, ovulation and spawning, but hCG had no effect on oocyte development before releasing eggs, but had an effect on latency period and time period of releasing eggs.

In conclusión, **i**t was found that the combined hormones of LHRH + hCG was the best treatment for brood stock in term of fertilization percentage. Therefore, this is an important step towards the domestication and culture of *Dicentrarchus labrax*, which is good aquaculture potential species.

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الحث الهرموني لتبويض اسماك القاروص البحرية

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جمعت أمهات أسماك القاروص من مزارع سمكية خاصة بمحافظة دمياط بمتوسط وزن ٢٠٠٠ ± ٢٠٠ جرام للذكور و٣٠٠٠ ± ٤٠٠ للإناث. تم نقل الأسماك إلى مزرعة خاصة بالقنطرة غرب – محافظة الإسماعيلية – مصر. إجمالي عدد الأمهات ٧٢ سمكة قاروص قسمت على أربع معاملات ولكل معاملة ٣ مكررات لتقييم أنتاج البيض تحت تأثير الحقن بالهرمونات المختلفة. تكونت المعاملة من ٤ ذكور و٢ إناث بنسبة جنسية ٢ :١ . تم حقن الأسماك خلال شهر يناير عندما كانت درجة حرارة الماء ١٤.٨°م. تم حقن الأربع معاملات (ذكور وإناث) كما يلي: المجموعة الأولى: (الكنترول) حقنت بمحلول ملحي ٠.٩%، المعاملة الثانية: حقنت ب ٢٥ مللي hCG /كجم وزن حي، والمعاملة الثالثة: حقنت ب ٥٠ مللي LHRH /كجم وزن حي، والمعاملة الرابعة: حقنت بجرعتان الأولى تم حقنها بـ ٢٥ مللي LHRH /كجم وزن حي في العاشرة صباحاً والجرعة الثانية تم حقنها بـ ١٢.٥ مللي hCG /كجم وزن حي بعد ٢٤ ساعة من الجرعة الأولى.

في هذه الدراسة سجلت أفضل النتائج من حيث نسبة الإخصاب في المعاملة الرابعة المحقونة بجرعتين الأولى ب LHRH والثانية hCG. هرمون LHRH يعالج الخلل الوظيفي للغدد الصماء الذي يحدث للأسماك في الأسر لإتمام عملية النضج النهائي للبويضات والتبويض والتفريخ بينما ال hCG غير مؤثر على تطور البويضات قبل عملية إطلاق البيض ولكن يؤثر على الفترة الزمنية قبل إطلاق البيض وفترة إطلاق البيض. ولذلك فهذه خطوة هامة لتطوير استزراع أسماك القاروص، وهى من الأنواع المرغوبة في الاستزراع البحري.