*Arab Gulf J. Scient. Res.*, **16** (2), pp. 403-414 (1998)

# Germ Cell Development in the European Eel, *Anguilla anguilla* L. during the Induction of Spermatogenesis with Human Chorionic Gonadotropin (HCG)

**E.M. Amin**

*Department of Ichthyology, The National Institute of Oceanaography and Fisheries, Alexandria, Egypt*

ABSTRACT. The migrating immature European freshwater eels, *Anguilla anguilla* L. undertake extensive migration from Egyptian lagoons to their spawning grounds in the Sargasso Sea. They leave their feeding grounds at an early stage of sexual maturation. Eels held in seawater were injected at weekly intervals with a human chorionic gonadotropin hormone (HCG) at a dose of 1.5 IU / gBW, and spermatogenesis was studied cytologically. The weekly injections have increased the gonadosomatic index (GSI) from 0.03 % to 9.0 %. The testicular maturation could be divided into five stages: a stage of seminiferous tubule differentiation (7 days of HCG treatment), a stage of the appearance of cysts of spermatogonia in the testes (15 days of HCG treatment), a stage of intensive spermatogenesis (3 weeks of HCG treatment), a stage of active testicular development (27 days of HCG treatment) and a stage of maximum sexual development (32 days of HCG treatment).

European freshwater eels, *Anguilla anguilla* L. undertake extensive migration from their feeding grounds in Egyptian lagoons towards their spawning grounds in the Sargasso Sea at an early stage of sexual maturation (Bertin 1956, Amin 1974, 1997 and Kohnenko *et al.* 1977). However, complete maturation of the testes of these eels may be induced by application of exogenous gonadotropins, and spermatogenesis has been described by several authors (Schreiber 1937, Tuzet and Fontaine 1937, Kokhnenko and Bezdenegnek 1975, Bieniartz *et al.* 1981, Pankhurst 1982 and Amin 1986).

The present study was undertaken to investigate the developmental changes and their synchronization with morphological changes during induced maturation induced by the injection of the human chorionic gonadotropin hormone (HCG).

## Materials and Methods

Fifty male silver eels, *Anguilla anguilla* L. (32.0-44.0 cm, length and 80-120 g, weight) were collected from Lake Edku (one of the Egyptian Northern Delta Lakes) during the period of spawning migration that extends from November to February. Experimental fish were gradually acclimatized to seawater for 7 days before the injection with HCG.

Thirty eels were injected intramusculary once each week with a human chorionic gonadotropin preparation (500 IU HCG), as described by Amin (1986). Eye index ($mm^2$) was calculated from the vertical x horizontal eye diameter measured with calipers, and was recorded together with the body colour before HCG injection. The remaining 20 eels were used as controls. At seven day intervals, four of the injected fish were killed to monitor gonadal development.

The testes were removed weighed, fixed in Bouin's and processed for histological examination according to the methods of Sakun and Butskaya (1968). Five to 10 μm thick serial paraffin sections were prepared from them and were stained with Mayer's haematoxylin and eosin.

The stages of testicular maturation were identified according to Yamamoto *et al.* (1972) and the gonadosomatic index (GSI) was determined.

## Results

The testis of the silver eel extends along the body cavity on each side of the gut. It appears as a series of pale pink, petal-shaped structure. Its average length is 10.0 ± 1.0 cm and its average width is 2.0 ± 0.5 mm. The average weight of the testis is 0.03 ± 0.005 g, with a gonadosomatic index (GSI) of 0.03 ± 0.005 %. The left lobe anterior to the urogenital opening is smaller than the right one. At the ripe stage (after 32 days of HCG injection), the average testis weight has risen to a massive 9.0 ± 0.9 g with a GSI of 9.0 ± 0.9 % (Fig. 1). All of the treated eels have shown noticeable external signs of maturation after four weeks of HCG treatment, such as the enlarged eyes with the iris surrounded by a dark blue ring and is associated with an increase in the pupil size (Fig. 2), together with dorsal bronze colouration, lateral metallic bronze and ventral metallic silver, with dark pointed pectoral fin. The characteristic odour of ripeness was given off by all of the fully matured eels.

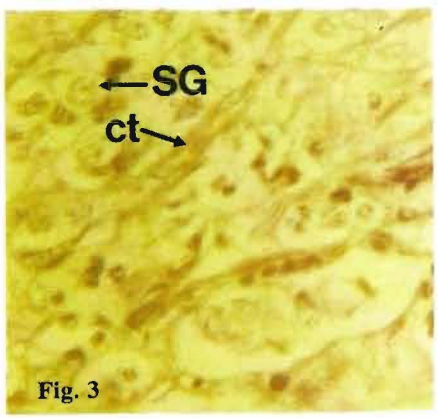
Fig. 1. Maximum development of the testes of a ripe European silver eel.

Fig. 2. Maximum eye-size of a ripe European silver eel.

The germinal tissues of the freshwater eels are formed as an extended network of spermatogonia separated by fibrous connective tissue. Each spermatogonium, which is spherical and has a central nucleus, is surrounded by somatic cells which may subsequently develop to form a cyst wall (Fig. 3). The spermatogonia are present indedendently of each other without forming any clusters, and they all appear in a resting phase.

In the testes of silver eels, several oocytes in the early peri-nucleolus stage could be detected intermingling with spermatogonia (Fig. 4). On the other hand, the testes of the controls contain well-developed seminiferous tubules with spermatogonia in

the resting phase and are arranged in cysts. Two types of somatic cells, besides blood and connective tissue cells, could be recognized. Sertoli cells are present within the seminiferous tubules, and Leydig cells in the intertubular spaces of the seminiferous tubules (Fig. 5).



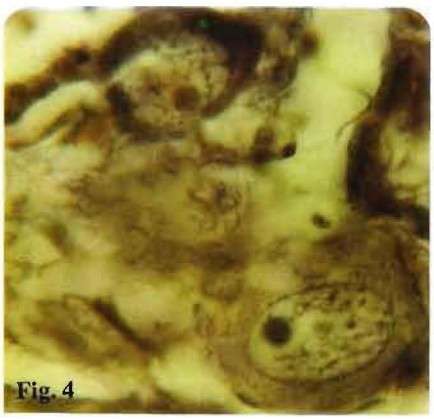
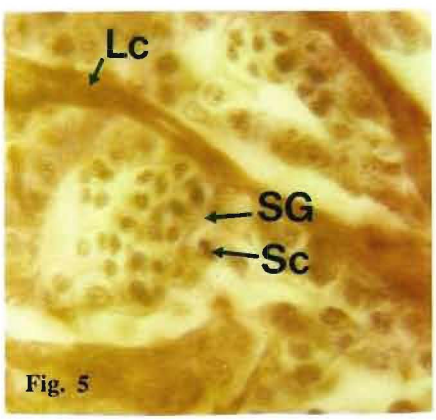




**Fig. 3.** Testis of an immature European silver eel. Spermatogonia (SG), connective tissue (ct) (X640).

**Fig. 4.** Oocytes in a testis of an immature European silver eel (X640).



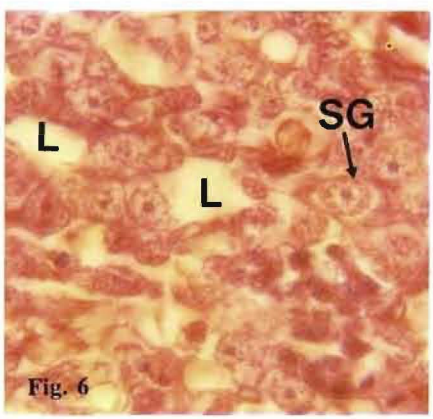




**Fig. 5.** Testis of a European silver eel acclimatized to seawater. The testis contains cysts of spermatogonia (SG), Sertoli cells (Sc) and Leydig cells (Lc)(X640).

**Fig. 6.** Testis with spermatogonia (SG) in stage I of maturation of the European silver eel. The seminiferous tubules are with central lumina (L)(X640).

Gradual changes in the testes were observed following treatment with HCG. Five stages could be identified:

### Stage I

Seven days after the initial injection, the seminiferous tubules become more differentiated each with a central lumen and spermatogonia were seen around the lumen singly or in clusters. The single ones are the largest in size and are characterized by distinct cytoplasm surrounding a large round nucleus, with the chromatin forming thick threads that extend uniformally over the nucleus (Fig. 6). On the other hand, the nucleus of the clustered spermatogonia is small and round, with many chromatin granules and many of the spermatogonia are in mitotic activity producing primary spermatocytes (Fig. 7).

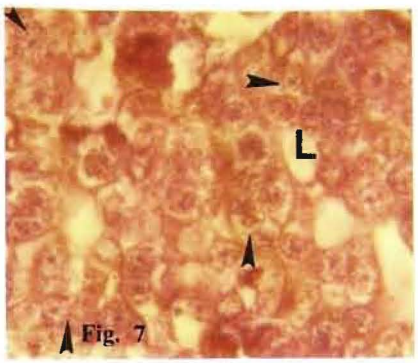
### Stage II

After 15 days of HCG treatment, the cysts of spermatogonia and primary spermatocytes could be seen surround; the narrow lumina. The lumen appeared to be lined with seminiferous epithelium continuous with the alveoli that developed in the peripheral germ cell cysts. The spermatogonia were large, occurred singly, and were few in number. The primary spermatocytes, seen on the inner margin of the tubules, were distinguished from spermatogonia by their smaller nuclei and darkly staining material (Fig. 8). They were further characterized by the aggregation of the chromatins to one pole of the nucleus. The Leydig cells were present as clusters of several cells; they had an elliptical nucleus and very thin cytoplasm.

### Stage III

Intensive spermatogenesis is evident at the end of the third week of HCG treatment at all stages of development (Fig. 9), and there were few isolated spermatogonia around the periphery of the tubules adjacent to the intertubular connective tissues. The predominant cell types were primary and secondary spermatocytes, although clusters of spermatozoa could be seen in the lumen.

Spermatids in young and final phases of spermiogenesis can be seen in Fig. 10. As maturation changes proceed, the cysts rupture and release spermatozoa into the tubule lumen. The spermatozoa aggregate into cluster. Sertoli cells, visible along the tubule wall, had round nuclei with a weak affinity to haematoxylin. The nuclei of the Leydig cells were round and had a comparatively strong affinity for haematoxilin.



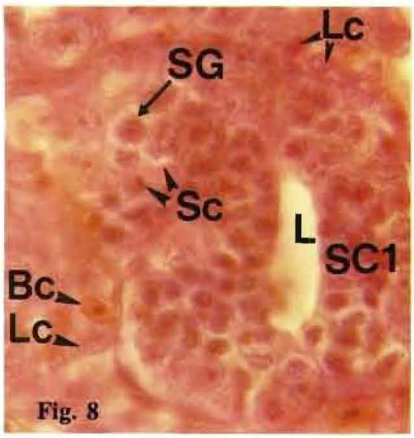
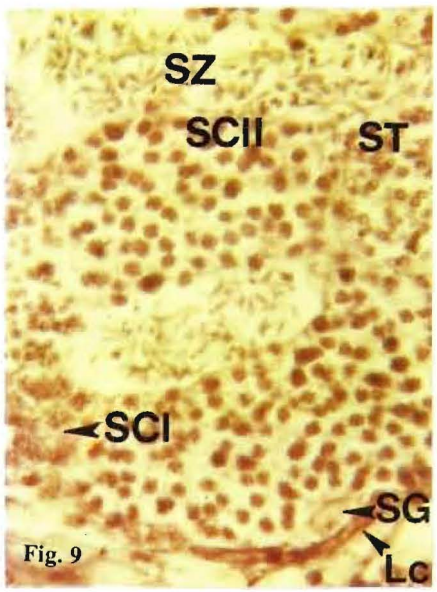




**Fig. 7.** Testis with clusters of spermatogonia in stage I of maturation (Arrowheads) of the European silver eel (X640).

**Fig. 8.** Testis in stage II of maturation of the European silver eel, showing seminiferous tubule with lumen (L), spermatogonia (SG), primary spermatocyes (SCI), Sertoli cells (Sc), Leydig cells (Lc) and blood cells (Bc)(X640).



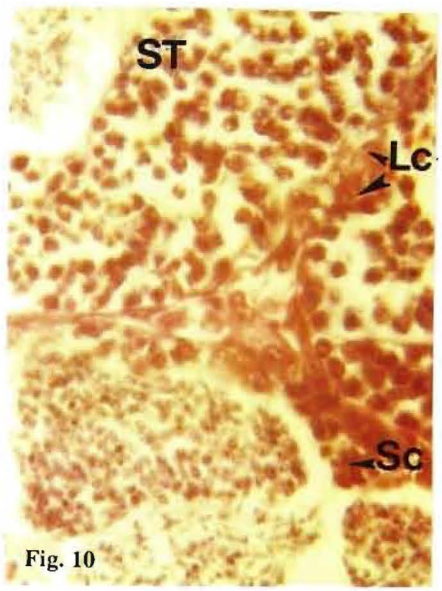




Fig. 9. Testis at the end of the third week of HCG treatment of the European silver eel, containing spermatogonia (SG), primary and secondary spermatocytes (SCI, SCII), spermatids (ST), spermatozoa (SZ)(X640).

**Fig. 10.** Spermatids at various phases of spermiogenesis in the European silver eel (X640).

### Stage IV

After 27 days of HCG treatment there were large numbers of spermatozoa in the lumen of the tubules. Spermatozoa could be distinguished by their elongated crescent-shaped bodies and dark staining chromatin. Small clusters of spermatids were also present (Fig. 11).

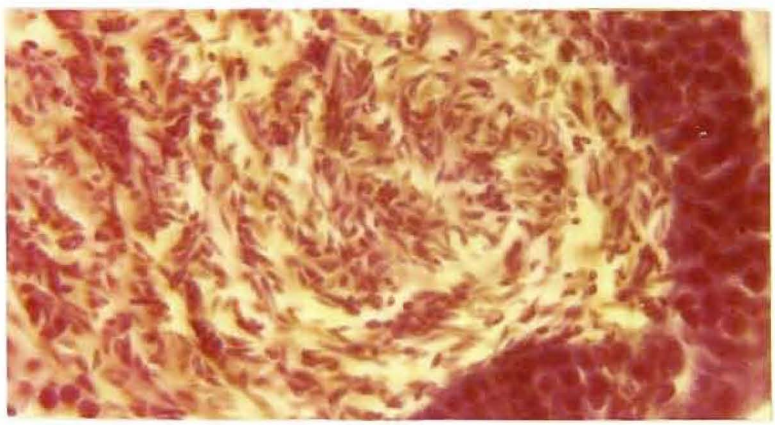


**Fig. 11.** Lumen packed with spermatozoa in the testis of the European silver eel (X800).

### Stage V

This stage of development was observed after five hormone injections (32 days of HCG treatment). Milt could be stripped exerting slight pressure on the abdomen to posterior direction. Spermatozoa remained motile in sea water for about 350 seconds.

## Discussion

Testicular development and maturity of the silver European eel, *Anguilla anguilla* L. does not occur before catadromous migration (Bertin 1956), but can be induced by hormonal injections (Amin 1986).

At the beginning of hormonal (HCG) treatment, the testes were immature and the most advanced cells they contain were spermatogonia. However, weekly injections of HCG have induced successive and gradual testicular maturation. Similar observations were also made in the Japanese eel (Yamamoto and Nagahama 1973, and Miura *et al.* 1991). Moreover, similar to the observations of Yamamoto and Nagahama (1973), mammalian gonadotropins were shown in the present study to induce testicular maturation in the European silver eel. Fish gonadotropins were

also proven to induce such maturation (Kawauchi *et al.* 1989, Planas *et al.* 1993, and Budworth *et al.* 1994).

Leydig cells were present in large numbers in the mature testes of piranha Myleus ternetzi (Loir *et al.* 1989). Changes in nuclear diameter and in the numbers of nuclei seemed to reflect various phases of the metabolic cycle in relation to spermatogenesis. Moreover, Yoshikuni and Nagahama (1991) have observed that Sertoli cells play an important role in the spermatogenesis of the Japanese eel.

Thus, adequate environmental factors, such as water quality, temperature and stocking density, together hormonal factors seem to be necessary for the testicular maturation in the European silver eel.

## References

**Amin, E.M.** (1974) *Morphological and biological studies on European eel* (*Anguilla anguilla* L.) *in the Egyptian waters.* Ph.D. Thesis, VNIRO Moscow, 100 p.

**Amin, E.M.** (1986) Induced gonadal maturation of male European eel (*Anguilla anguilla* L.) inhabiting the Egyptian lakes. *Arab Gulf J. Scient. Res.* **4:** 293-301.

**Amin, E.M.** (1997a) Gonad differentiation and early gonadal development of the European eel *Anguilla anguilla* L. in Egyptian water. *Arab Gulf J. Scient. Res.* **15**(1): 175-186.

**Bertin, L.** (1956) *Eel. A Biological Study.* Cleaver-Hume Press, London, 185 p.

**Bieniartz, K.P., Epler, P., Malczewski, B.** and **Passakas, T.** (1981) Development of European eel (*Anguilla anguilla* L.) gonads in artificial conditions. *Aquaculture* **22:** 53-66.

**Budworth, P.R., Senger, P.L., Griswold, M.D.** and **Donaldson, E.M.** (1994) Relationship of plasma steroids to germ cell development and the presence of protamine mRNA in rainbow trout during the induction of spermatogenesis with partially purified salmon gonadotropin. *J. Fish Biol.* **44:** 983-995.

**Kawauchi, H., Suzuki, K., Itoh, H., Swanson, P., Naito, N., Nagahama, Y., Nozaki, M., Nakai, Y.** and **Itoh, S.** (1989) The duality of teleost gonadotropins, *Fish Physiol. Biochem.* **7:** 29-38.

**Kokhnenko, S.V.** and **Bezdenegnek, B.A.** (1975) Stimulation of gonadal maturation of male European eel by gonadotropin hormone. *J. Fish Econ.* **1:** 26-28.

**Kokhnenko, S.V., Bezdenegnek, B.A.** and **Gorovaya, S.L.** (1977) Ecologophysiological plasticity of European eel. *Sci. Technic. Minsk,* 93-98 pp.

**Loir, M., Margeridon, A.** and **Cauty, C.** (1989) Leydig cells in *Myleus ternetzi* testes. *Aquat. Living Resour.* **2:** 57-61.

**Miura, T., Yamauchi, K., Takahashi, H.** and **Nagahama, Y.** (1991) Hormonal induction of all stages of spermatogenesis *in vitro* in the male Japanese eel (*Anguilla anguilla* L.). *Proc. Nation, Acad. Sci.* **88:** 5774-5776.

**Pankhurst, N.W.** (1982) Relation of visual changes to the onset of sexual maturation in the European eel *Anguilla anguilla* (L.) *J. Fish Biol.* **21:** 127-140.

**Planas, J.V., Swanson, P.** and **Dickhoff, W.W.** (1993) Regualtion of testicular steroid production *in vitro* by gonadotropins (GTH-I and GTH-II) and cyclic AMP in coho salmo (*Oncorhynchus kisutch*). *Gen. comp. Endocrinol.* **91:** 8-14.

**Sakun, O.F.** and **Butskaya, M.** (1968) Determination of the stages of maturation and studies of sexual cycle in fishes. *PINRO, Mormansk. USSR.* **2:** 5-23.

**Schreiber, B.** (1937) Ricerche sulla spermatogenesi accelerata nell'anguilla. *Archo. Zool. Ital.* **24:** 147-167.

**Tuzet, O.** and **Fontaine, M.** (1937) Sur la spermatogenese de l'anguille argentee. *Arch. Zool. Exp. Gen.* **78:** 199-215.

**Yamamoto, K., Hiro, O., Hirano, T.** and **Morroka, T.** (1972) Artificial maturation of cultivated male Japanese eel by synahorin injection. *Bull. Jap. Soc. Sci. Fish.* **38:** 1083-1090.

**Yamamoto, K.** and **Nagahama, Y.** (1973) Cytological changes in the adenohypophysis of fresh-water cultivated male Japanese eels, *Anguilla japonica,* induced to maturation by transfer to sea water and Synahorin injection. *Bull. Jap. Soc. Sci. Fish.* **39:** 585-594.

**Yoshikuni, M.** and **Nagahama, Y.** (1991) Endocrine regulation of gametogenesis in fish. *Bull. Inst. Zool. Acad. Sin. Monogr.* **16:** 139-172.

*(Received 01/03/1997;*
*in revised form 01/06/1998)*

# علاقة الهرمون المنشط للمناسل الإنساني (Human Chorionic Gonadotropin) بنمو الخلايا المنبتة في خصي ثعبان السمك الأوروبي (*Anguilla anguilla* L.) خلال التكوين المنوي

إكرام محمد أمين

*المعهد القومي لعلوم البحار والمصايد - الإسكندرية - مصر*

عند الإستعداد لهجرة ثعبان السمك الأوروبي من البحيرات الشمالية المصرية للتوالد في بحر السرجاسو في المحيط الأطلنطي (نحو ٤٠٠٠ كم) ظهرت مناسل تلك الأسماك ضامرة وهي في مراحلها الأولى للنضوج .

كان الهدف الأساسي من هذه الدراسة هو معرفة علاقة الهرمون المنشط للمناسل الإنساني بتطور ونمو مناسل ثعبان السمك الأوروبي حتى النضوج التام ، ودراسة المراحل المختلفة للخلايا الجنسية أثناء مراحل نموها المختلفة . لقد أجريت التجارب على ذكور ثعبان السمك الفضي وبإستخدام الحقن الهرموني أسبوعياً ولمدة ٣٢ يوماً ، ويمكن تلخيص النتائج التي تم الحصول عليها كما يلي :

- ذكور الأسماك التي جمعت من المياه العذبة تميزت بمناسل في حالة نمو مبكر جداً واحتوت مناسلها على بعض الخلايا الجنسية الأنثوية وهي أيضاً في مراحل تكوينها الأولية .
- عند أقلمة الأسماك في مياه البحر وقبل استخدام الحقن بالهرمون بدأ واضحاً بعض التطور الملحوظ في المناسل ، إذ تكونت الأنابيب الجنسية ، أما أمهات

المنـي (Spermatogonia) فظلت كـما هي في حـالة السكـون ولكن في شكل مجاميع بعدها ظهر نوعان من الخلايا الجنسية وهي خلايا سرتولي (Sertoli Cells) الموجـودة داخـل الأنبـيـبـات المنوية (Seminiferous tubules) أمـــــا الأخرى فهي خلايا ليدج (Leydig Cells) المنتشرة بين تلك الأنبيبيات .

- بعد سـبعـة أيام من الحقنة الأولى ظهـرت الأنبيـبيات المنوية بوضـوح وبهـا التجاويف واتخذت أمهات المني مراحل مختلفة من التطور .
- بعد ١٥ يوماً من الحقن ونتيجة تضـاعف ثم نمو أمـهات المني بدأ النوع الثاني من الخـلايا الجنسيـة المنوية في الـظهـور ، في حين قلت أعـداد أمـهـات المني وفي الوقت نفسه ظهرت خلايا ليدج في شكل مجاميع .
- في نهـاية الأسبـوع الثالث من الحـقن (بعـد ثلاثة مرات حـقن) أمكن الحصـول على مناسل بـها جـميع مـراحل التطور المختلفـة إلى جـانب ظهـور مـجـاميع من الحبيبات المنوية في تجـويف الأنبيبيات المنوية وأيضاً ظهـرت خلايا سـرتولي وليدج وبأعداد كبيرة .
- بعـد ٢٧ يومـاً ، أي بعـد الحـقن الرابـع ظهـرت أعـداد كـبيـرة من الحـبيـبـات المنوية تتخللها بعض مجاميع من الحبيبات المنوية غير المكتملة التكوين (Spermatids) .
- بعـد الحقن الخـامس (بعد ٣٢ يومـاً) أمكن الحصـول بمجرد الضـغط على بطن السمكـة على حيوانات منـوية ظلت تسبح بنشـاط لمدة ٣٥٠ ثانية في مياه من البحر .

ولقـد اتضح من هذه الـدراسـة أن للـهـرمـون المنشط للـمناسل الإنسـاني (Human Chorionic Gonadotropin) تأثيـره في وصـول معـدل نمو المناسل إلى ٩٪ والحصول على الحيوانات المنوية التي يمكن استخدامـها في تكاثر هذا النوع الهام جداً من الأسماك .