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Gonad Differentiation and Early Gonadal Development of the European Eel *Anguilla anguilla* L. in Egyptian Waters

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ABSTRACT. Sex differentiation and gonadal development in the fresh water European eel. *Anguilla anguilla* L. was histologically studied for elvers, young and adults. The sex groups were divided into undifferentiated elver and adult and definite female or male. The successive examinations of the eel's gonads revealed that the primordial germ cells first appear in 6.4-10.0 cm elvers and the oogonia in 15.0 cm ones. Elvers destined to become females differentiate sexually at a length beyond 19.0 cm and those destined to become males beyond 27.0 cm, but some adult fish 38.0 cm in length still have undifferentiated gonads. Hermaphrodite gonads occur at a length of 32.0-35.0 cm. The ovary is the main gonad that directly develops, while the testis plays the role of secondary sex development. The oocytes proliferate rapidly, but the testis enters a period of quiescence. Both sexes possess gonads in immature conditions, the oocytes at the previtellogenic stage and spermatogonia in late multiplication stage. The presence of a *vas deferens* during gonadal development signals testicular differentiation, and its absence leads to ovarian development.

Few adequate histological descriptions of early gonadal development, germ cell morphology, and time of sex differentiation in the European eel, *Anguilla anguilla* L. have been undertaken (Rodolico 1933, D'Ancona 1943, Frost 1945, Svardson 1949, Sinha and John 1966, Amin 1974 and Colombo *et al.* 1984). Similar to the Japanese eel (Takahashi and Sugimoto 1978) it is known to exhibit a juvenile hermaphroditism (Rodolico 1933, D'Ancona 1950, Bertin 1956, and Colombo *et al.* 1984). However, basic information about the early gonadal development, germ cell

morphology and time of sex differentiation during the life cycle of this fish are still laking.

Hence, the present study have been undertaken to provide that basic information, as well as to delineate the way in which eel gonads differentiate.

Materials and Methods

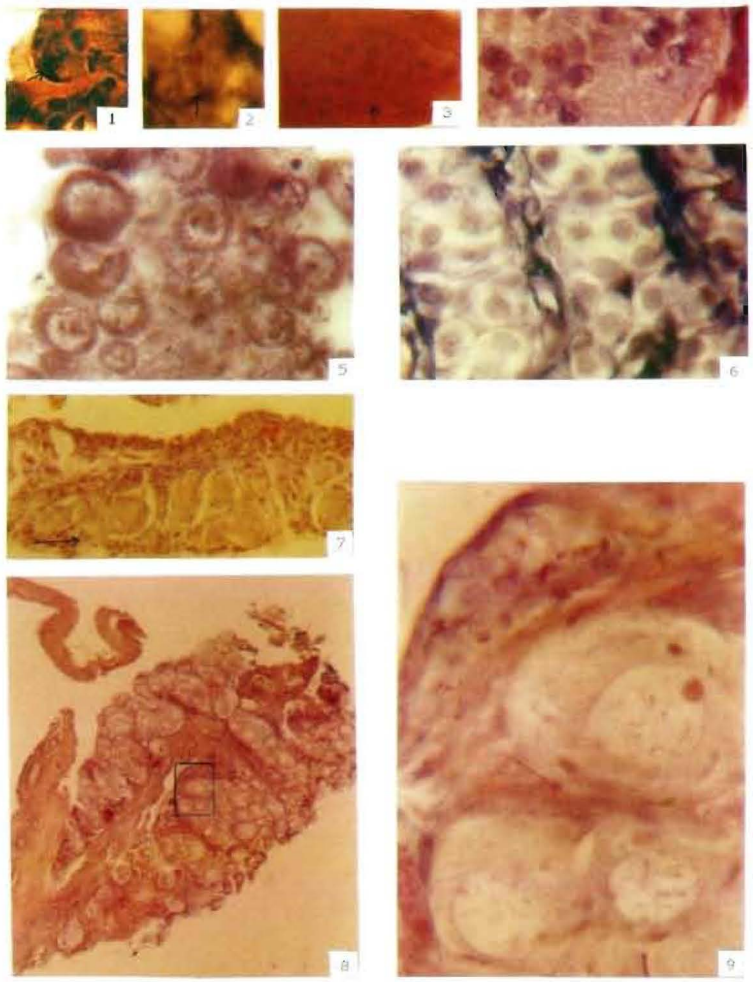
A total of 100 eels were used in the present study. They were obtained from lake Edku (one of the Egyptian northern delta lakes). The eels were maintained in the laboratory into fresh water aquaria. They included 20 elvers, 6-12 cm in length; 40 juveniles 14-22 cm long; 20 yellow eels, 20-40 cm long and 20 silver eels, 30-50 cm in length.

The abdominal segments obtained from elvers and the gonads from juveniles and adults were fixed in Bouin's fixative for 24-48 h and were processed for histological examination using standard techniques. Then 5-10 μm thick serial paraffin sections were cut from them, stained with Mayer's haematoxylin and eosin (H & E) and their histological structures were observed under the microscope. The developmental stages of oocytes were identified according to the description Yamamoto *et al.* (1974).

Results

Undifferentiated and differentiated gonads were detected. The former occur in virtually all elvers and in some adults, and the latter in young and adult females or males. The gonads of 6.4 cm elvers are undifferentiated and contain primordial germ cells between the mesonephric duct and the gut (Fig. 1). Cysts containing few germ cells occur beneath the dorsal peritoneal wall in 8.3 cm elvers (Fig. 2). On the other hand, the germ cells of the gonads of larger elvers (10.0 cm) are actively multipling giving rise to smaller cells (Fig. 3). The undifferentiated gonads were also found in some adult fish 38.0 cm long with abundant germinal tissues that contain many gonial germ cells (Fig. 4), some of which were degenerating (Fig. 5). In another individual (Fig. 6), the germ cells are round or ovoid with clear cytoplasm and centrally located small nucleoli. The sex of such fish could not be identified.

Gradual change from an undifferentiated state to a definite female was first observed in 15.0 cm long fish where mitotic configurations in germ cells giving rise to nests of oogonia (Fig. 7) were encountered. In 19.0 cm long fish, nests of oogonia that have originated from the peripheral ridge of the ovary become organized into



Figs. 1, 2 and **3.** Transverse sections of undifferentiated gonads of 6.4, 8.3 and 10.0 cm elvers, respectively. Arrows indicate primordial germ cells (X520).

Figs. 4 and **6.** Undifferentiated gonads in 38.0 cm long eels (X 520).

Fig. 5. Degenerating oogonia in 38 cm long fish (X 520).

Fig. 7. Transverse section of a gonad of a 15 cm fish. Arrow shows nests of oogonia (X 208).

Fig. 8. Ovary of a young 19 cm eel. Arrow shows the *vas deferens* (X 208).

Fig. 9. Higher magnification of the boxed area in Fig. 8. Note oogonia with large nuclei (X 520).

groups (Fig. 8) and through rapid multiplication and growth give rise to large, round morphologically uniform oogonia, each with a single prominent nucleolus (Fig. 9).

Larger individuals (23.0 cm long) have ovaries densely packed with oocytes which contain condensed chromatin in wider lamellae (Fig. 10). Gonadal lamellae that contain oocytes (Fig. 11) are, however, formed in larger fish samples (35.0 cm long).

The ovarian development is characterized by two growth phases: a phase occurring in yellow eels' ovaries comprising gonad encompassed cells with prominent nuclecoli, indistinguishable cell outline and colourless cytoplasm surrounding dark nuclear membrance (Fig. 12). A second phase occurring in silver eels' ovaries and comprises cells with disproportionately large nuclei (each about 50 μm in diameter) that contain dense chromatin granules (Fig. 13). These are accompanied in yellow eels by peri-nucleolus basophilic stage where the spherical enlarged nucleus occupies most of the cell and contains numerous acidophilic, irregularly-shaped nucleoli (Fig. 14). The oocysts are characterized by homogeneous basophilic cytoplasm that changes to deep blue or purple black at the initiation of lipogenesis. The average egg diameter at this stage is 80 μm (Fig. 15), which then enlarges to an average diameter of 90 μm (Fig. 16) after the eels have changed from yellow to silver. Thereafter, few yolk vesicles appear in the outer zone of the oocytes' cytoplasm. These oocytes are almost round, less darkly stained, with large vacuoles and have an average diameter of 108 μm (Fig. 17). The most advanced stage in the development of silver eels' ovaries comprises oocytes with yolk globules accumulating in perinuclear cytoplasm that contains numerous vacuoles. The nuclei decrease in volume and the eggs measure 137-177 μm with a mean diameter of 160 μm (Fig. 18).

Definite males are not easy to recognize before the eels have attained a total length of 27.0 cm. The germinal tissues are formed as extended network of rows composed of germ cells separated by sheets of fibrous connective tissues (Fig. 19). In larger specimens (29.0 cm long), such rows are bordered by fibromuscular connective tissues containing fibroblasts and flattened cells. While the spermatogonia are enveloped with cytoplasmic sheets of follicle cells that have flat, rod-shaped nuclei (Fig. 20). All germinal cells, however, transform into spermatogonia in the testes of 30.0 cm long fish and they appear in either resting or mitotic phases (Fig. 21). Following the transformation from yellow into silver eels, the spermatogonia become round in shape with centrally located, dense nuclei and are arranged in cysts (Fig. 22). However, the principal feature of a definite male is the appearance of a prominent *vas deferens* (Fig. 23) in the testes of 32.0 cm long

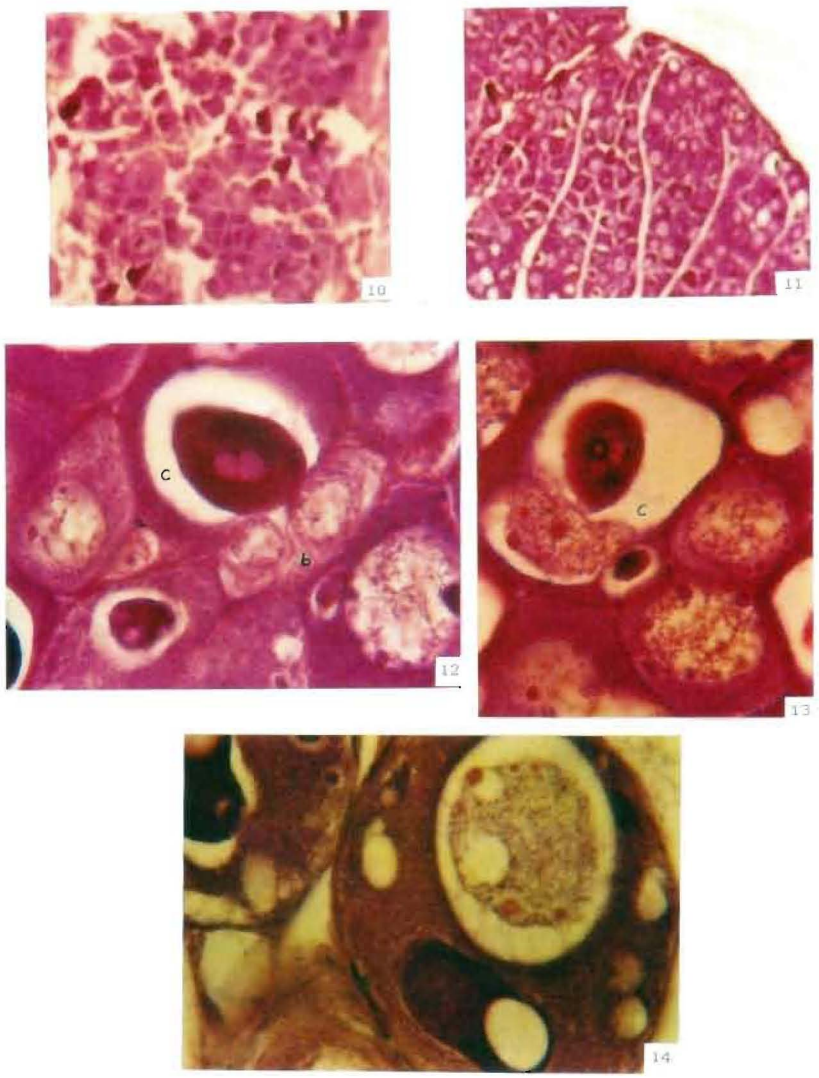


Fig. 10. Cross section of an ovary of a 23 cm long fish (X 208).

Fig. 11. Cross section of an ovary of a 35 cm long fish (X 208).

Fig. 12. A transverse section of a developing ovary; a) smallest cell visible at this stage; b) cell with gossamer-like reticulum; c) cell with dense chromatin nucleus (X 520).

Fig. 13. Oocytes in early peri-nuclear stages (X 520).

Fig. 14. Oocytes in peri-nucleolus basophilic stages (X 520).

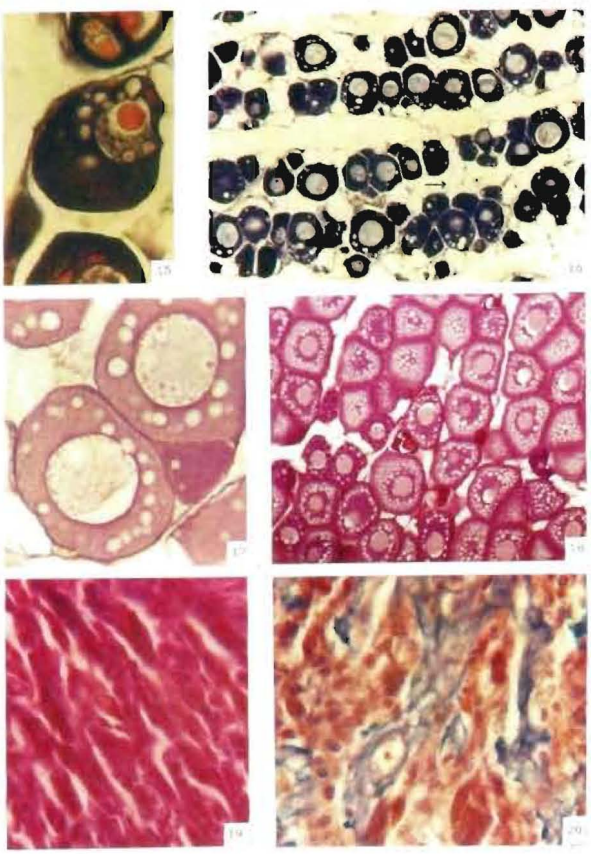


Fig. 15. Ovary of a yellow eel (X 164).

Fig. 16. Section of ovaries of silver eels (X 164).

Fig. 17. Primary yolk globule stage of an ovary of a silver eel (X 164).

Fig. 18. Advanced stage of ovarian development, showing yolked eggs with darkly stained yolk globules throughout cytoplasm (X 41).

Fig. 19. Testis with large lamellae of a 27 cm long eel (X 410).

Fig. 20. Germinal rows in a 29 cm long eel (X 410).

fish. Moreover, at this stage of fish development, hermaphrodite gonads where oocytes intermingle with spermatogonia (Fig. 24) can be detected in the testes of male eels. These oocytes are surrounded by somatic cells (Fig. 25) and some of them are degenerate with pycnotic nuclei and vaculated cytoplasm.

Hermaphrodite gonads also occur in 35.0 cm long female yellow eels where discrete, compact spermatogonia do not intermingle with oocytes, but are localized along the distal edge of the ovigerous lamellae. Some of the spermatogonia are in the resting phase, while others are degenerate (Fig. 26).

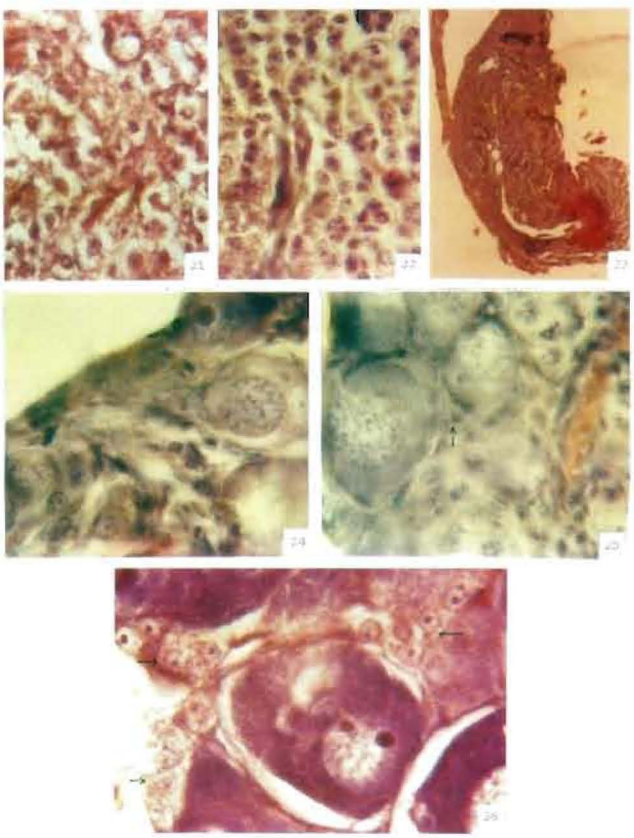


Fig. 21. Resting spermatogonia of an immature yellow eel (X 410).

Fig. 22. Testis of a silver eel in late multiplication (X 410).

Fig. 23. Testis of silver eel with *vas deferens* (X 164).

Fig. 24. Testis of an immature yellow eel containing oocytes (X 410).

Fig. 25. Oocytes enclosed by somatic cells (arrow), in a testis of an immature yellow eel (X 410).

Fig. 26. Ovary of an immature eel. Arrows show testicular cysts filled with spermatogonia in resting stage (X 410).

Discussion

The results indicate that the onset of sexual differention in the European eel. *A. anguilla* mainly depends upon attaining a certain body length, but not on its age. The succesive two groups of male and female gonads are of variale duration according to the condition of the individual. Fishes of 6-12 cm in length (elvers) are immature hermaphrodites, 15 to 26 cm long ones are immature females, and those above 26 cm in length are either definite males or continue as females. Very few large eels, 38.0 cm in length have undifferentiated gonads. Hence, the gonads of the European eel seem to be essentially ovaries and appear in fishes 15 cm long.

Testicular tissues were absolutely absent till the fish becomes 27 cm long. Similar observations were made by Bertin (1956), but the opposite was reported by Wachtel and Koo (1981) who found that the yellow eel testis is the basic gonad which develops first and becomes an ovary due to the expression of H-Y antigen. However, the length at which eels attain sexual differentiation was found by several authors to be variable in the same, as well as in different species of eel (D'Ancona 1943, Frost 1945, Svardson 1949, Sinha and John 1966, Kuhlmann 1975, and Colombo *et al.* 1984).

The hermaphrodite phase of gonadal development is remarkable and is usually a temporary one that might extend into the adult eels. However, similar to the observations of Takahashi and Sugimoto (1978) in the Japanese eel, *Anguilla japonica,* the presence of ovarian tissues in testes or *vice versa* does not prevent the development of the opposite germ cells.

The histological examination undertaken in the present study has delineated the general appearance of various gonad developmental stages, especially in females, and the presence of spermatogonia and oocytes is considered as a mark of sexual differentiation. Both sexes of the eel possess the gonads in quite immature conditions at the commencement of their catadromous migration.

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تمييز المناسل والنمو المبكر لها في ثعبان السمك الأوروبي *Anguilla anguilla* L. في المياه المصرية

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يقوم ثعبان السمك الأوروبي *Anguilla anguilla* بهجرة طويلة وشاقة حيث يقطع مسافة قدرها حوالي ٤٠٠٠ كم تبدأ من البحيرات الشمالية المصرية مروراً بالبحر الأبيض المتوسط حتى يصل إلى بحر السرجاسو في المحيط الاطلنطي حيث يتم التوالد ، وهناك يموت الآباء بعد ظهور جيل جديد يسبح إلى موطن الآباء .

كان الهدف الأساسي من هذا البحث معرفة أساسيات النمو المبكر لمناسل صغار تلك الأسماك بعد وصولها للبحيرات الساحلية المصرية وكيفية التمييز الجنسي بين المناسل وتحديد الطول الذي تتميز عنده الأسماك إلى إناث أو ذكور ثم دراسة الصفات المورفولوجية للخلايا الجنسية منذ بداية تكوينها وحتى الطور اليافع الذي تتكرر عنده هجرة التوالد .

من الدراسات الهستولوجية الحالية على مناسل الزريعة والأسماك الصغيرة واليافعة إتضح أنه يمكن تقسيم مناسل تلك الأسماك إلى مجموعتين ، مجموعة غير متميزة الجنس وهذه واضحة في الزريعة وبعض الأسماك اليافعة ، أما المجموعة

الأخرى فهي متميزة الجنس إلى إناث وذكور .

لقد ظهرت الخلايا الجنسية البدائية لأول مرة في زريعة الأسماك التي تراوحت أطوالها من ٤ ,٦ إلى ١٠ سم في حين بدأ ظهور البيض عند طول ١٥ سم . وكان تمييز صغار تلك الأسماك جنسياً إلى إناث عند طول ١٩ سم في حين تميزت الذكور عند طول ٢٧ سم ، بينما ظلت مناسل بعض الأسماك اليافعة (٣٨ سم) في حالة عدم تمييز ، أما المناسل الخنثوية فقد ظهرت في بعض الأسماك التي تتراوح أطوالها من ٣٢ إلى ٣٥ سم . يشكل المبيض العضو الأساسي لتكوين الخلايا الجنسية في حين يبدو أن دور الخصية دور ثانوي ، والدليل على ذلك سرعة إنقسام البيض على عكس الخلايا الجنسية المذكرة التي عادة ما تدخل في مرحلة السكون لدى كل من الجنسين من الأسماك اليافعة . يتوقف نمو بيوض الأنثى عند مرحلة ما قبل التكوين المحي ، أما خصي الذكور فتحتوي على أمهات مني في مراحلها الأخيرة من التضاعف . تبدأ المناسل خنثوية وظهور الوعاء الناقل *(vas deferens)* يشير إلى تحولها للذكورة وإختفاؤه دليل على إكتساب صفة الأنوثة .