Acta Biologica Hungarica 56 (1–2), pp. 169–172 (2005)

# THE TESTIS HISTOLOGY OF ARTIFICIALLY MATURATED EUROPEAN EEL (*ANGUILLA ANGUILLA* L.) AT THE END OF SEXUAL MATURATION, AND SPERMATOZOA ULTRASTRUCTURE IN FRESHWATER REARING

## SHORT COMMUNICATION

T. Müller,[1,2]* F. Baska,[3] C. Niklesz,[3] P. Horn,[2] B. Váradi[1] and M. Bercsényi[1]

[1] Georgikon Faculty of Agriculture, University of Veszprém, Deák F. u. 16, H-8360 Keszthely,
[2] Faculty of Animal Science, University of Kaposvár, Guba S. u. 40. H-7400 Kaposvár,
[3] Faculty of Veterinary Science, Szent István University, István u. 2, H-1400 Budapest, Hungary

(Received: January, 15, 2004; accepted: March 12, 2004)

The artificial induction of sexual maturation of European eel males was carried out by using weekly hCG administrations. Histological pictures showed that the testis tissues developed and regressed naturally and no pathological changes took place under the conditions of artificial rearing in freshwater. According to light and electron microscopic investigations the morphology and motility of the spermatozoa of males kept in freshwater proved to be similar to those in seawater. The authors suppose that freshwater rearing of males is not a barrier factor in the artificial propagation of European eels.

*Keywords:* Sexual maturation – European eel – spermatozoa

Based on early observation it was supposed that eels die after spawning. In laboratory experiments males survived hormone treatments [1] and repeated spermiations could be induced when the males were fed between the treatment periods [3]. Previous results clearly revealed the unique ultrastructure of eel spermatozoa, which include a crescent-shaped nucleus, a rootlet attached to the neck region, a flagellum of the 9 + 0 pattern, and a pseudo-flagellum extending from the proximal centriole [4, 6]. The goals of the present study were to look into the testis histology in this critical period and to clarify whether the salinity of the rearing water influences the spermatozoal microstructure. Three groups ($w = 101 \pm 30.9$ g), each consisting of 3 males were formed and reared in freshwater. The first group served as control (group one), the second was given 250 IU human chorion gonadotropin (hCG)/fish/week for 8 weeks (group two) and the third group was given 250 IU hCG/fish/week for 19 weeks (group three). The males of group one were killed at the beginning of the experiment, group two on the 9th week and group three on the 27th week, two

*Corresponding author; e-mail: muller-t@georgikon.hu

Abbreviations: *dc* distal centriole, *dS* degenerated spermatozoa and spermatids, *Ct* connective tissue, *E* erythrocytes, *f* flagellum, *L* lumina, *m* mitochondrium, *n* nucleus, *pc* proximal centriole, *r* rootlet, *S* spermatozoa, *Sg* spermatogonium, *Sc1* primary spermatocyte, *Sc2* secondary spermatocyte, *St* spermatid

*0236-5383/$ 20.00 © 2005 Akadémiai Kiadó, Budapest*

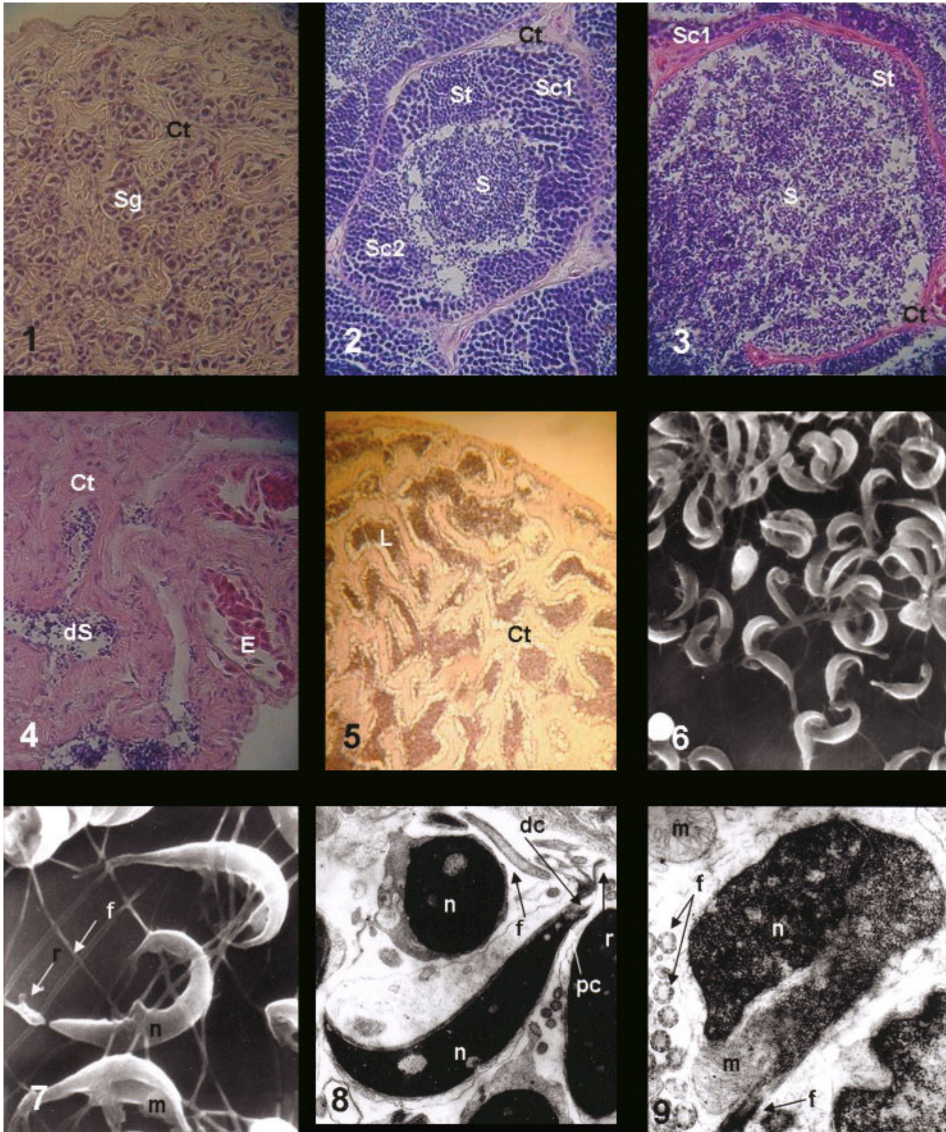


*Fig. 1.* Testis cross-section of untreated male. Spermatogonic tissue with many spermatogonia. The trabecula of the testis interstitium composed mainly by elastic and collagenic connective tissue elements (GSI = 0.07%), ×400. *Fig. 2.* Cross-section of a fully mature testis (GSI = 3.15%), ×400. *Fig. 3.* Matured spermyocytes in the lumina of the testes parenchyma surrounded with connective tissue (yellow) and smooth muscle (red) cell elements (7th fish, GSI = 8.11%), ×400. *Fig. 4.* Testis cross-section of 8th fish (GSI = 0.64%), ×400. *Fig. 5.* The tissue of the testis after release of the sperm. The trabecula of the testis interstitium composed by collagenic connective tissue elements (9th fish, GSI = 0.53%, Van-Gieson), ×100. *Fig. 6.* Scanning electron micrograph of mature eel spermiocytes. SEM, ×1300. *Fig. 7.* Scanning electron micrograph of mature eel spermiocytes. SEM, ×5300. *Fig. 8.* Longitudinal section of a mature spermiocyte. The head is intensive electron dense. TEM, ×5200. *Fig. 9.* Cross-section of the head of an eel spermyocyte with the chromatin and a part of the tubular mitochondrium. TEM, ×13 200

months after their last hormone treatment. Pieces of testes were fixed in 4% formaline, embedded in paraffin and 5 μm thick slices were stained with heamatoxylin-eosin for light microscopic observations. For the electron microscopic investigations small pieces of testes were fixed in Na-cacodylat buffered 2.5% glutardialdehyde, postfixed in buffered 1% osmium tetraoxide, embedded in Durcupan ACM. Ultrathin sections were made and examined with a JEOL 100 S electron microscope. The sperm obtained from the male eels was examined both in transmission and scanning electron microscopy.

The motility of the sperm sample was estimated at ×400 magnification using an Olympus CO11 microscope. One drop of artificial seawater (3.5% NaCl solution) was dropped onto a glass slide and ~1 μl sperm was mixed with it.

The investigated features of the testes of control (immature GSI (gonad weight/body weight × 100) = 0.07 ± 0.01%) and treated (midmature GSI = = 3.54 ± 0.47%) groups were similar as it was described earlier [2, 5] (Figs 1, 2). In group three 179 days after the first injection and 45 days after the last one the testes showed two different histological stages. On the wall of tubules of the 7th fish (GSI = 8.11%) there were some clusters of spermatocytes and spermatids and the tubules were full of spermatozoa. A large amount of blood vessels appeared in the connective tissue which is rich in smooth muscle elements. The 8th fish (GSI = 0.64%) and the 9th one (GSI = 0.53%) possessed thicker connective tissue than the number 7. They had smaller tubules, which contained only a small amount of spermatids and spermatozoa showing degenerated forms. The number of their erythrocytes increased compared to the 7th fish, which showed macroscopically visible hyperaemia at the necropsy. Apart from the spermatozoa there were Leydig and/or other somatic cells within the connective tissue. The various cell types are not easy to identify by common histological techniques [2]. In the testes of the hormonally treated eels an increased amount of smooth muscle could be detected by Van Gieson's staining. The ultrastructure of the mature spermiocytes showed no differences in comparison to naturally matured cells described in the literature (Figs 6–9, Table 1) [6]. The cytoplasm of the mature spermatozoa contained one large tubular mitochondrium at the apical part of the cell. The chromatin of the cells was highly electron dense, showing no fine structure, except a certain granulation.

The histological pictures showed that the testis tissues regressed naturally and no pathological changes took place under the conditions of artificial rearing either in freshwater (present study) or in seawater [1]. Males reared in freshwater can produce

*Table 1*
Data about the spermatozoa (*n = 50)

| Head length (μm) | Head width (μm) | Flagellum length (μm) | Rootleth length (μm) | Mitochondrion diameter (μm) | Sources |
|---|---|---|---|---|---|
| 5.4 (±0.4) | 1 (±0.2) | 25 (±5.5) | 1.2 (±0.2) | 0.8 (±0.3) | Okamura et al., 2000 |
| 5.6 (±0.5) | 0.9 (±0.3) | 26 (±0.7) | 1.2 (±0.3) | 0.8 (±0.4) | Present study* |

morphologically the same spermatozoa as "seawater males". The spermatozoa could be activated (10–90% motility) in artificial seawater. This is promising with regard to the multiple use of males. Since there is no significant difference between the morphology and motility of freshwater and seawater spermatozoa, the authors assume that the freshwater rearing of males is no limiting factor in the artificial propagation of European eels.

## REFERENCES

1. Boëtius, I., Boëtius, J. (1967) Studies in the European Eel, *Anguilla anguilla* (L.). Experimental induction of the male sexual cycle, its relation to temperature and other factors. *Medd. Dan. Fisk. Havunders. 4,* 339–405.
2. Colombo, G., Grandi, G., Romeo, A., Giovanni, G., Pelizzola, D., Catozzi, L., Piffanelli, A. (1987) Testis cytological structure, plasma sex steroids, and gonad cytosol free steroid receptors of heterologous gonadotropin (hCG)-stimulated silver eel, *Anguilla anguilla* L. *Gen. Comp. Endocrin. 65,* 167–178.
3. Dollerup, J., Graver, C. M. (1985) Repeated induction of testicular maturation and spermiation, alternating with periods of feeding and growth in silver eels, *Anguilla anguilla* (L.). *Dana 4,* 19–39.
4. Gibbons, B. H., Gibbons, I. R., Bacceti, B. (1983) Structure and motility of the 9 + 0 flagellum of eel spermatozoa. *J. Submicr. Cytol. 15,* 15–20.
5. Hibiya, T. (1982) *An Atlas of Fish Histology.* Kodansha, Tokyo, Gustav-Fischer-Verlag, New York, Stuttgart.
6. Okamura, A., Zhang, H., Yamada, Y., Tanaka, S., Horie, N., Mikawa, N., Utoh, T., Oka, H. P. (2000) Re-examination of the spermatozoal ultrastructure of eels: observations of the external morphology of spermatozoa in three species. *J. Fish Biol. 57,* 161–169.