Chapter 2

Sequence of Germ Cells Differentiation During Spermiogenesis of the Amphibian Urodele *Ambystoma dumerilii*

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Abstract

The spermatogenesis, including the spermiogenesis, in Urodeles contains the meiotic process and the morphological differentiation of the spermatids developing the spermatozoa as in the rest of vertebrates. However, in Urodeles, there are essential differences in the structure of the testis, as a lobular structure; the distribution of the spermatogenic cells, in cephalocaudal progression in the testis; and the cystic condition of the developing spermatogenic cells in synchronous groups bounded by Sertoli cells. All the spermatogenic cells are situated in parallel position with the heads directed to the same side. The big size and elongated morphology of the spermatozoa also characterized this type of spermiogenesis. Spermiation occurs at the caudal portion of the testis to the efferent duct system, which includes the mesonephric nephrones.

Keywords: lobular testis, longitudinal spermatogenesis, spermiogenesis, testicular cysts, Urodeles

1. Introduction

The spermatogenesis of Urodeles occurs in longitudinal course into the testis. The structure of the testis forms abundant longitudinal lobules which contain the germinal cells. The lobules are separated by trabeculae of thin and vascularized connective tissue, which are the continuation of the tunica albuginea. The spermatogonia are situated in the cephalic edge of the testis, and the development of spermatozoa occurs during the way of the spermatogenesis through the testicular lobules to the caudal edge of the testis (**Figure 1A–C**). At the end of the lobules, the spermatozoa are discharged to the deferent duct system [1, 2]. Consequently, the disposition of spermatogenesis in Urodeles is longitudinal, in cephalocaudal progression, where the

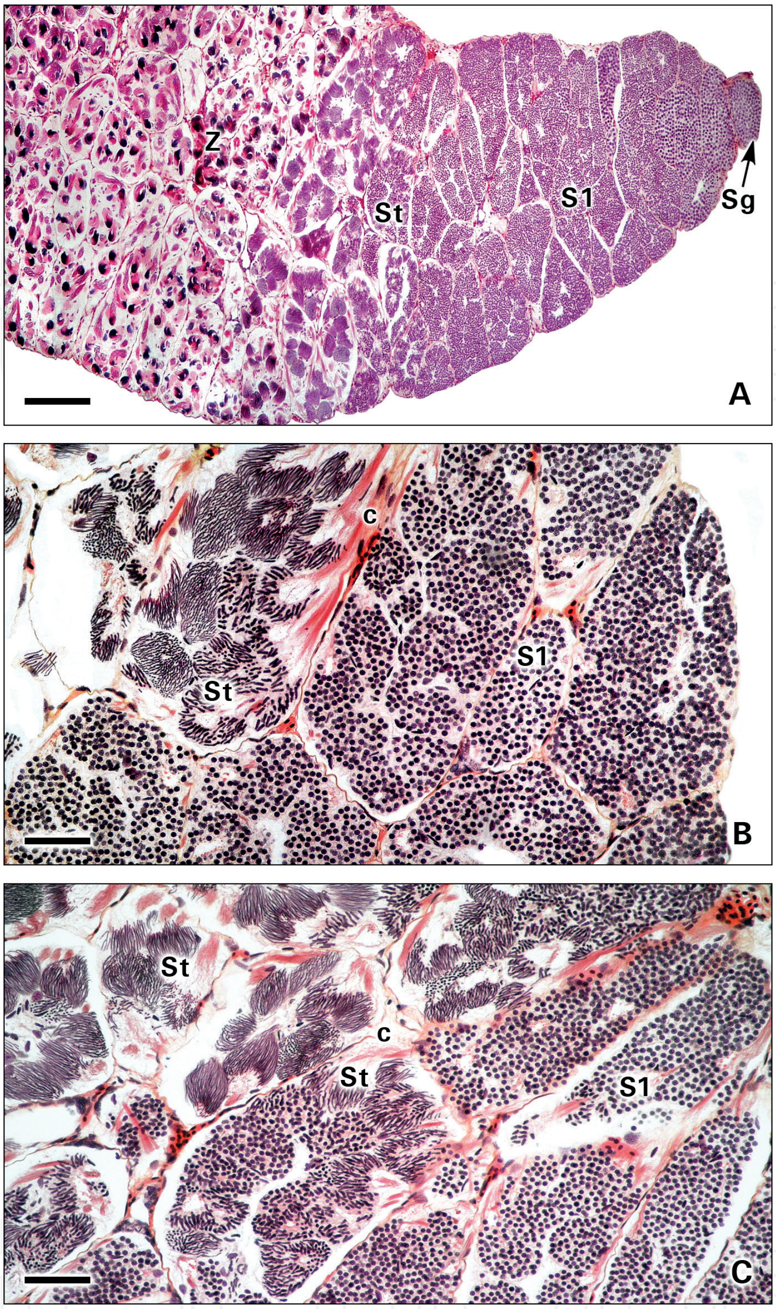

Figure 1. Testis of *Ambystoma dumerilii* in longitudinal sections. Spermatogenesis advancing in successive regions along the cephalocaudal axis of the testis, where the different types of spermatogenic cells are seen. (A) Panoramic morphology of the testis. H-E. Bar = 0.3 mm. (B, C) Testicular lobules containing the spermatogenic cells and surrounded of connective tissue. H-E. Bar = 0.1 mm. Spermatogonia (Sg), primary spermatocytes (S1), spermatids (St), spermatozoa (Z), and interlobular connective tissue (c).

earliest stages are more cephalic and the latest stages are more caudal, in contrast with the tubular structure with radial disposition of the spermatogenesis in the testis of amniotes.

Spermatogenic cells of Urodeles are quite big, compared to amniotes germ cells, as example, the spermatogonia may attain 55 μm in *A. dumerilii* [1, 3] and the spermatozoa may attain 840 μm long in *Necturus maculosus* [4, 5].

For the description of this type of spermiogenesis of Urodeles, we consider convenient detailed illustration in this chapter of the progressive histological changes of the spermatids during the development of the spermatozoa, taking the species *Ambystoma dumerilii* (Ambystomatidae) as a model. The histological sections were stained with hematoxylin-eosin (H-E), Masson's trichrome, periodic acid-Schiff (PAS), and alcian blue. *A. dumerilii* is an endemic species, which habits at the southern edge of the Mexican Plateau in Michoacán State, Mexico, in the Lake Pátzcuaro (260 km^2, moderately shallow to 11 m, and high elevation at 2035 m up sea level). *A. dumerilii* is a neotenic species, because lack metamorphosis, maintaining during all the life cycle as paedomorphic aquatic larva [6].

2. Spermatogenesis in Urodeles

Spermatogenesis in Urodeles was studied by several authors who described stages of germ cell maturation in a variety of species as: in *Desmognathus fusca* [7]; in *Ambystoma tigrinum* [8–10]; in *Trituroides hongkongensis* [11]; in *Necturus maculosus* [12]; in *Salamandrina terdigitata* [13]; in *Salamandra salamandra* [14, 15]; in *A. mexicanum* [16, 17]; Ricote et al. in *Triturus marmoratus* [18]; in *A. dumerilii* [1, 3, 17, 19]; and in *Salamandrella keyserlingii* [20].

The spermatogenic cells of *A. dumerilii*, as in all Urodeles, are in synchronous groups called cysts, where all the cells are at the same stage of development. A cyst is formed when a spermatogonium becomes surrounded by a Sertoli cell. Then, the distribution of cysts in the testicular lobules displays a longitudinal sequence of stages of spermatogenesis, in respect to the cephalocaudal gradient: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids in spermiogenesis, and spermatozoa. The Sertoli cells are involved in essential functions of the spermatogenesis: they maintain a permeability barrier to the germinal cells into the cyst during all the process of differentiation, determine the endocrine activity that controls the spermatogenesis, and phagocytose degenerating spermatogenic cells, residual bodies, and abnormal spermatozoa during the spermiogenesis [1, 17, 19].

Spermatogonia of *A. dumerilii* are spherical cells with 45–55 μm in diameter (**Figure 2A**). These cells are diploid and have mitotic activity. When spermatogonia initiate the meiotic process become a primary spermatocyte (**Figure 2A**–**C**).

The primary spermatocytes are also spherical cells; their size is 40–45 μm in diameter. These cells initiate the meiosis; then, their nuclei contain duplicated chromosomes at different stages of prophase I of meiosis exposed clearly in the chromatin changes: leptotene with fine reticular chromatin, zygotene with fine fibrillar pattern of duplicated chromosomes, pachytene with more thick fibrillar pattern of duplicated chromosomes in crossing-over, and diplotene when occurs the separation of homologous duplicated chromosomes, remaining some chiasms (**Figure 3A** and **B**). The primary spermatocytes enter metaphase I, anaphase I, and telophase I (**Figure 3C**), resulting in two secondary spermatocytes.

Secondary spermatocytes are spherical cells and are smaller than primary spermatocytes; they have in average 18–20 μm in diameter. As the result of the first division of meiosis, the secondary

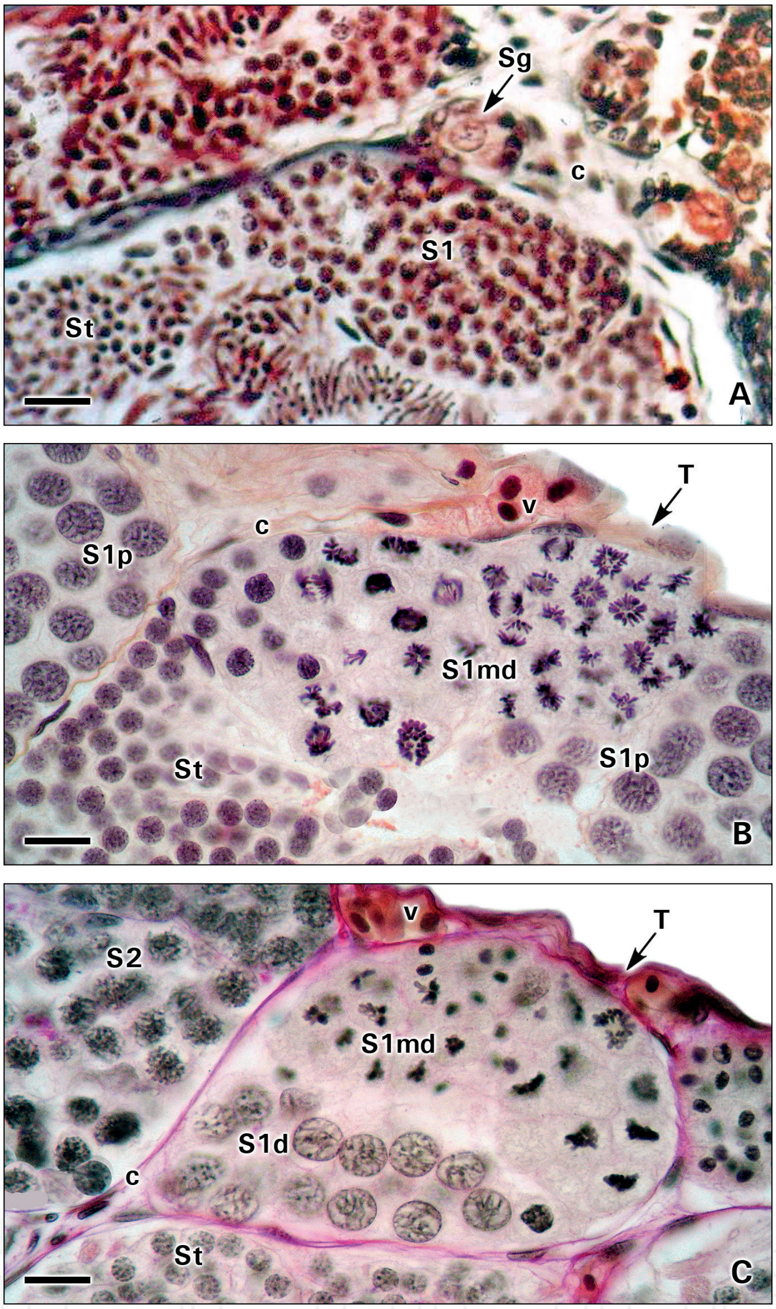

Figure 2. Spermatogenesis in the testis of *Ambystoma dumerilii*. (A) Cephalic region of the testis. Spermatogonia surrounded by connective tissue. Lobules with cysts containing cells in different stages of spermatogenesis. Masson's trichrome. Bar = 30 μm. (B, C) Periphery of the testis in the adjacent region to spermatogonia. Cysts containing primary spermatocytes during the first meiotic prophase, in pachytene, with thick fibrillar chromatin, in diplotene with pairs of chromosomes showing chiasms and during the first meiotic division, secondary spermatocytes and early spermatids. Around the lobules, there is connective tissue. The tunica albuginea with blood vessels surrounds the testis. Spermatogonia (Sg), primary spermatocytes (S1), primary spermatocytes in pachytene (S1p), primary spermatocytes in diplotene (S1d), primary spermatocytes during the first meiotic division (S1md), secondary spermatocytes (S2), spermatids (St), connective tissue (c), tunica albuginea (T), and blood vessels (v). B: H-E, C: PAS. Bar = 20 μm.

spermatocytes contain a haploid, but duplicated, number of chromosomes (**Figure 2C**). These cells are seen less frequent, since they divide during the second part of meiosis after a very short interphase, rapidly giving rise to two spermatids.

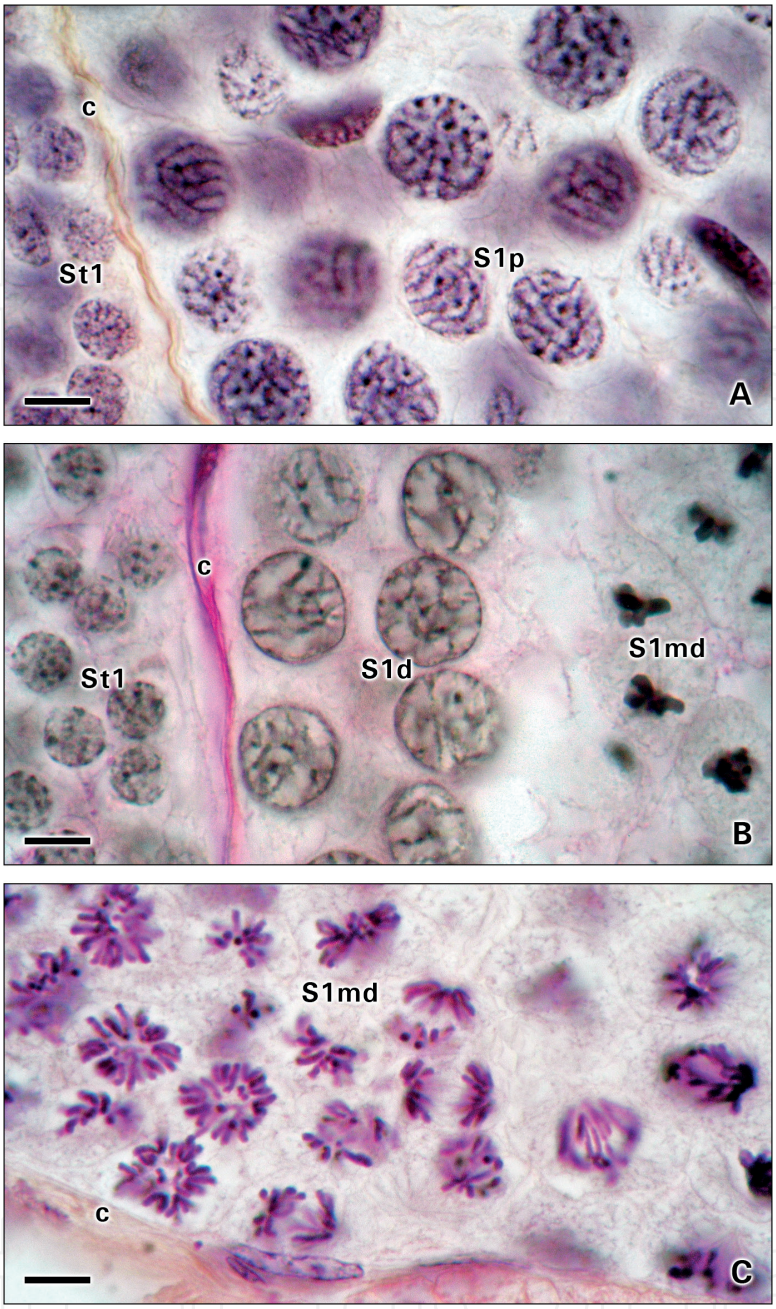

Figure 3. Spermatogenesis in the testis of *Ambystoma dumerilii*. (A) Primary spermatocytes during pachytene and early spermatids. Compare the size of both type of germinal cells. H-E. Bar = 10 μm. (B) Primary spermatocytes during diplotene and early spermatids. Compare the size of both type of germinal cells. PAS. Bar = 10 μm. (C) Primary spermatocytes during the first meiotic division. B: H-E, C: PAS. Bar = 10 μm. Primary spermatocytes in pachytene (S1p), primary spermatocytes in diplotene (S1d), primary spermatocytes during the first meiotic division (S1md), early spermatids (St1), and connective tissue (c).

3. Morphology of spermatids in *A. dumerilii* during spermiogenesis

The spermatids of *A. dumerilii* initiate the spermiogenesis, occurring during a sequence of morphological changes transforming the spermatids into spermatozoa. Early spermatids are spherical in shape and attain a diameter of 14–17 μm; their nuclei contain light fibrillar chromosomes.

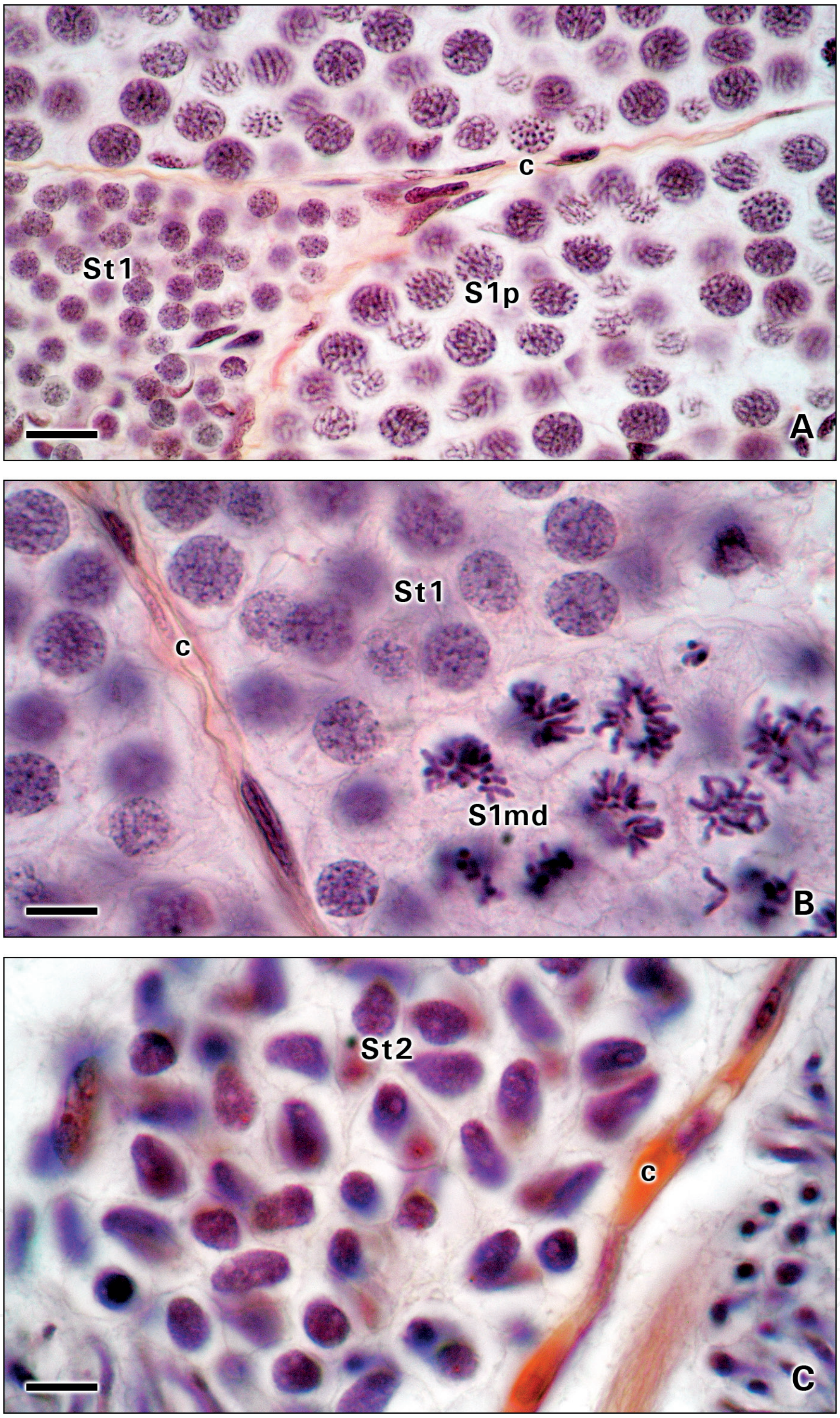

Figure 4. Early spermatids in the testis of *Ambystoma dumerilii*. (A) Primary spermatocytes during pachytene and early spermatids with round nucleus. H-E. Bar = 20 μm. (B) Early spermatids with fine fibrillar chromatin. H-E. Bar = 20 μm. (C) Initial elongation of the spermatids and more compact aspect of the nucleus. H-E. Bar = 20 μm. Early spermatids (St1), (St2) and interlobular connective tissue (c).

The early spermatid nuclei soon are seen as fine granular and progressively come to dense. The early spermatids become progressively elongated and the chromatin shows increasing degree of condensation (**Figure 4A–C**). As spermiogenesis proceeds, the nuclei of spermatids become

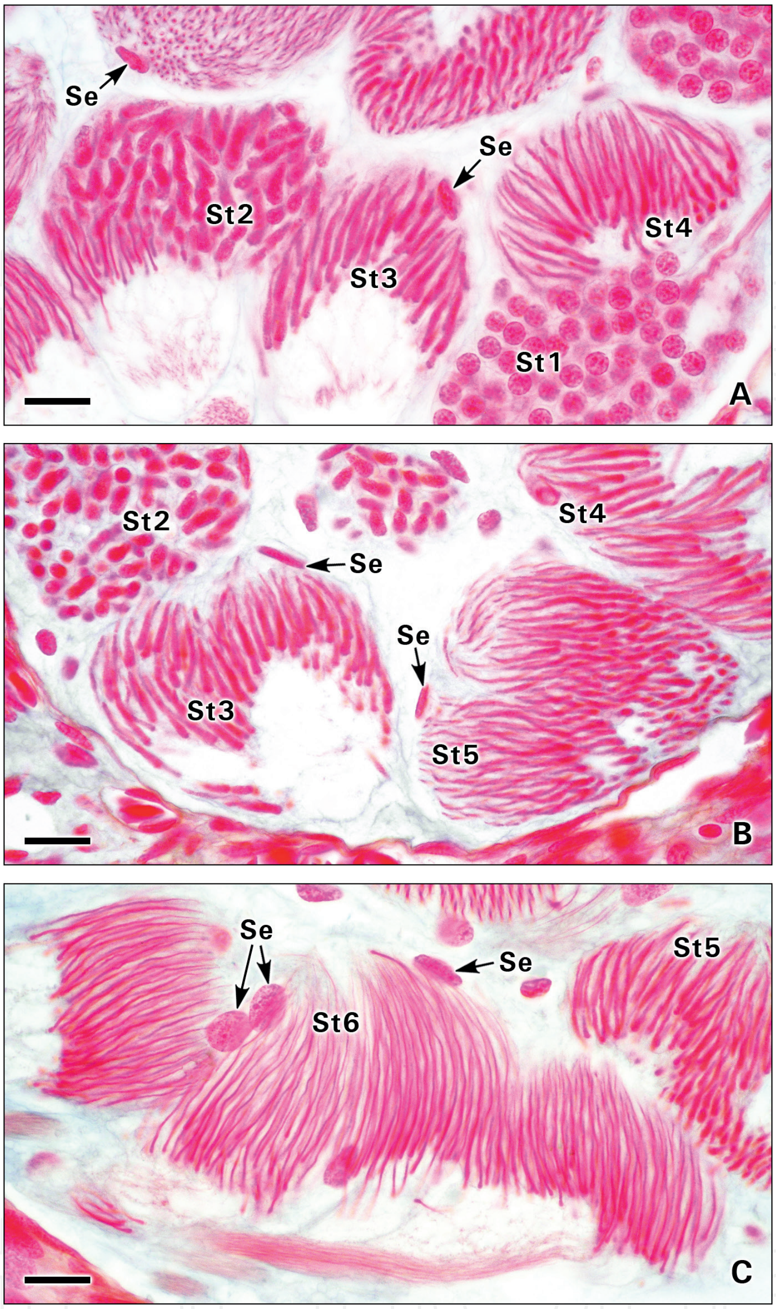

Figure 5. Spermiogenesis in the testis of *Ambystoma dumerilii*. (A–C) Early spermatids, when they are spherical (1). Various cysts present the evident progressive elongation of the spermatids. The nuclei of Sertoli cells are seen around the cysts. Alcian blue. Bar = 20 μm. Early spermatids (St1). Spermatids in elongation (St2), (St3), (St4), (St5), and (St6). Sertoli cells nuclei (Se), connective tissue (c).

larger (**Figures 5A–C** and **6A–C**). The shape of spermatids in spermiogenesis is gradually performing an elongated cell developing head, midpiece, and flagellum. These three parts of the cell are clearly distinguished, additionally to their shape and position in the cell, because their different staining affinity: the head is basophilic; the midpiece is intensely acidophilic; and the flagellum is also acidophilic but less intense than the midpiece. The head of the spermatozoa contains the acrosome and the nucleus, with a narrower cephalic part at the acrosome. All the germinal cells in a cyst maintain the same orientation, with the heads to the same side

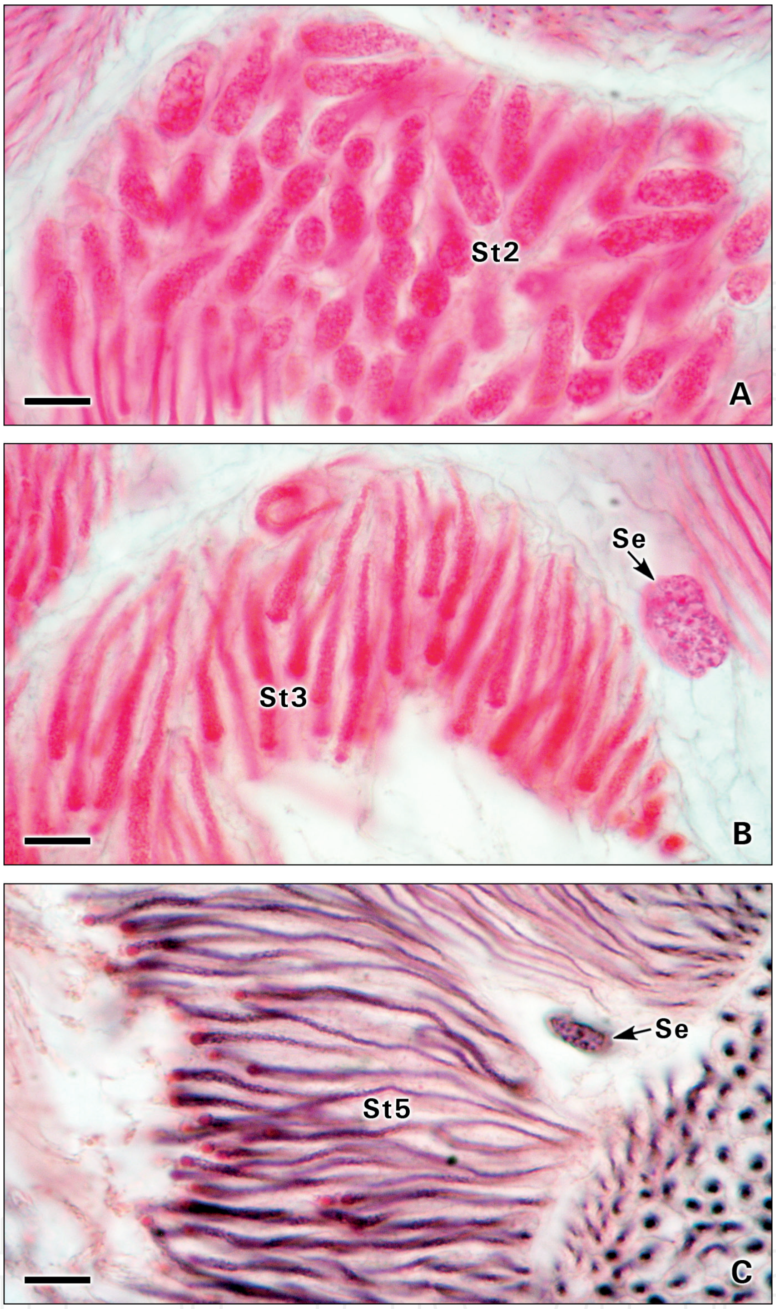

Figure 6. Spermiogenesis in the testis of *Ambystoma dumerilii*. Details of the progressive elongation of the spermatids. (A–C) Cysts contain spermatids during the progressive elongation. Progressive elongation of spermatids (St2), (St3), and (St5). Sertoli cells nuclei (Se). (A, B) Alcian blue. (C) H-E. Bar = 10 μm.

(**Figure 7A–C**). As maturation advances the spermatozoa have a swirl arrangement inside the cyst, keeping their heads oriented in the same direction (**Figure 8A–C**). The large of the spermatozoa may attain 460 μm [1, 6].

The total length of spermatozoa of Urodeles is usually longer than those of other amphibians and other vertebrates. The shortest spermatozoa were reported for *Hynobius nebulosus* with a length of 156 μm, whereas the longest, as we documented before, with a length of 840 μm, was observed in *Necturus maculosus* [4, 5]. The lengths of spermatozoa differ

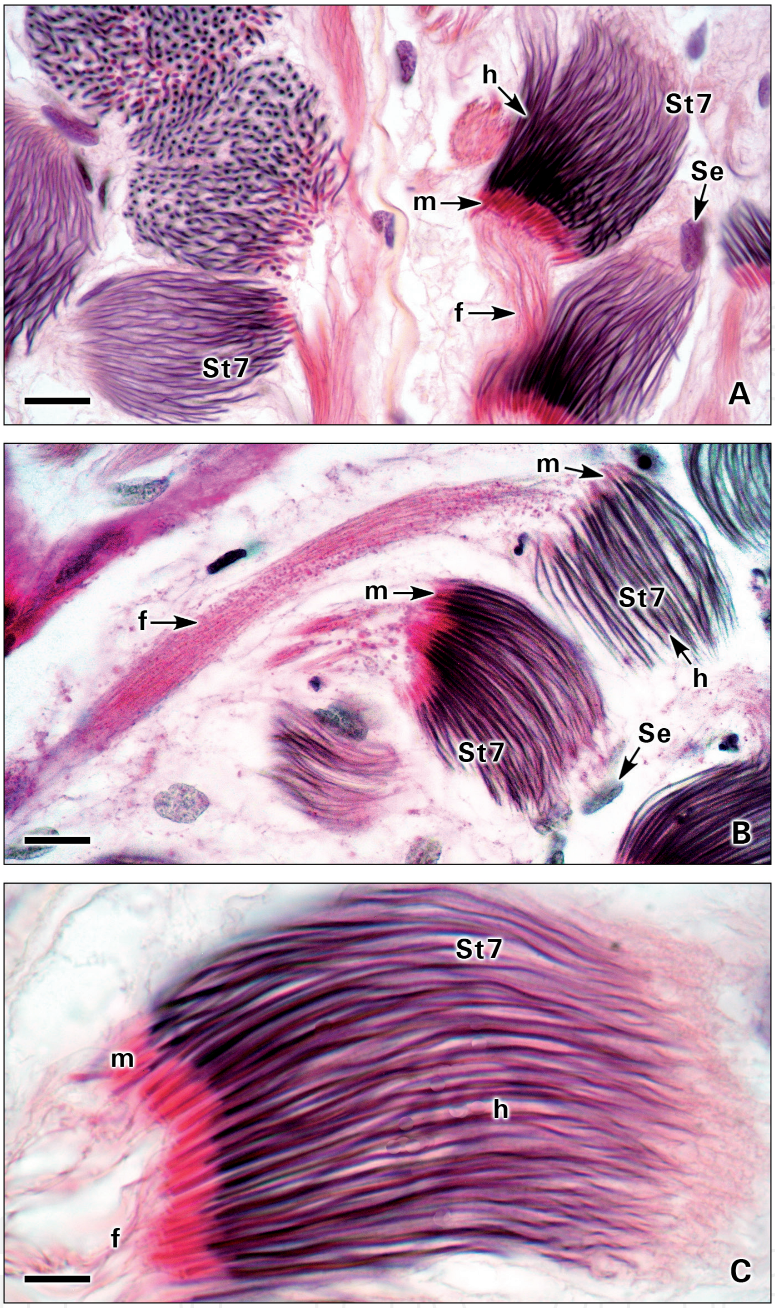

Figure 7. Spermiogenesis in the testis of *Ambystoma dumerilii*. (A–C) The elongation of the late spermatids advances into the cysts. The development of the head, the midpiece, and the flagella is observed by the different staining affinity. The cells conserve the same position, with the heads directed to the same side. (A) H-E. Bar = 20 μm. (B) H-E. Bar = 20 μm. (C) H-E. Bar = 10 μm. Spermatids (St7), head (h), midpiece (m), flagella (f), and Sertoli cells nuclei (Se).

widely in Urodeles as examples are: *Hynobius boulengeri* (197 μm); *Salamandrella keyserlingii* (212 μm) [21]; *Lissotriton italicus* (360 μm) [22]; *Desmognathus aeneus* (388 μm) [23]; *Ambystoma mexicanum* (444 μm) [6]; *Eurycea bislineata* (459 μm), *E. lucifuga* (523 μm), *Pleurodeles dorsalis* (536 μm), *P. dunni* (626 μm) [23]; *Triturus helveticus* (650 μm) [4]; and *Aneides aeneus* (770 μm) [23]. The biological significance of the differences in the lengths of spermatozoa is unknown.

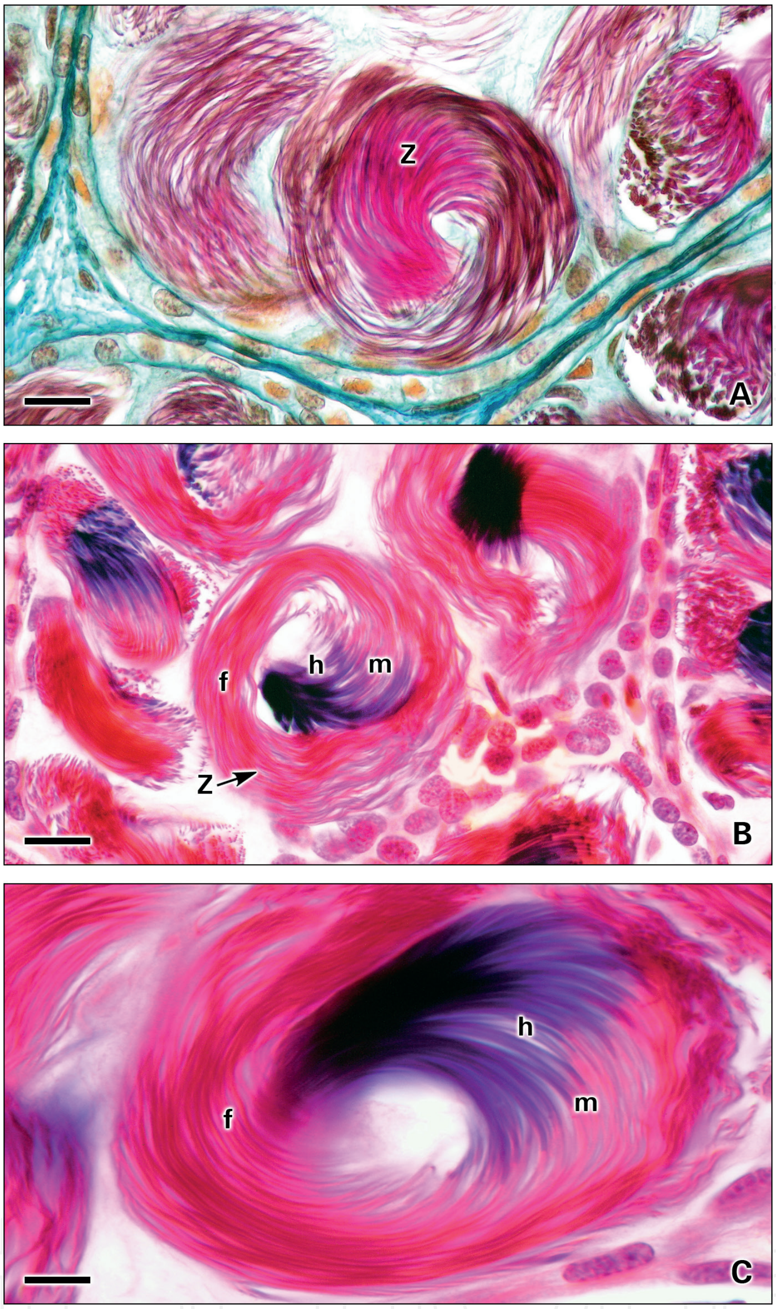

Figure 8. Spermatozoa in the testis of *Ambystoma dumerilii*. (A–C) The cellular maximum elongation is observed in the spermatozoa, and additionally, the spermatozoa screw in where the head is in the interior part and the flagella in the exterior part of this roll, maintaining the cystic condition. (A) Alcian blue. Bar = 20 μm. (B) H-E. Bar = 20 μm. (C) H-E. Bar = 10 μm. Spermatozoa (Z), head (h), midpiece (m), and flagella (f).

4. Spermiation

The region of spermiation is observed at the caudal end of the testis, where the density of cysts with spermatozoa decreases, there are abundant empty cysts containing remnants of Sertoli cells, and few cysts containing spermatozoa, compared with the region before spermiation where there are abundant cysts with spermatozoa (**Figure 9A** and **B**).

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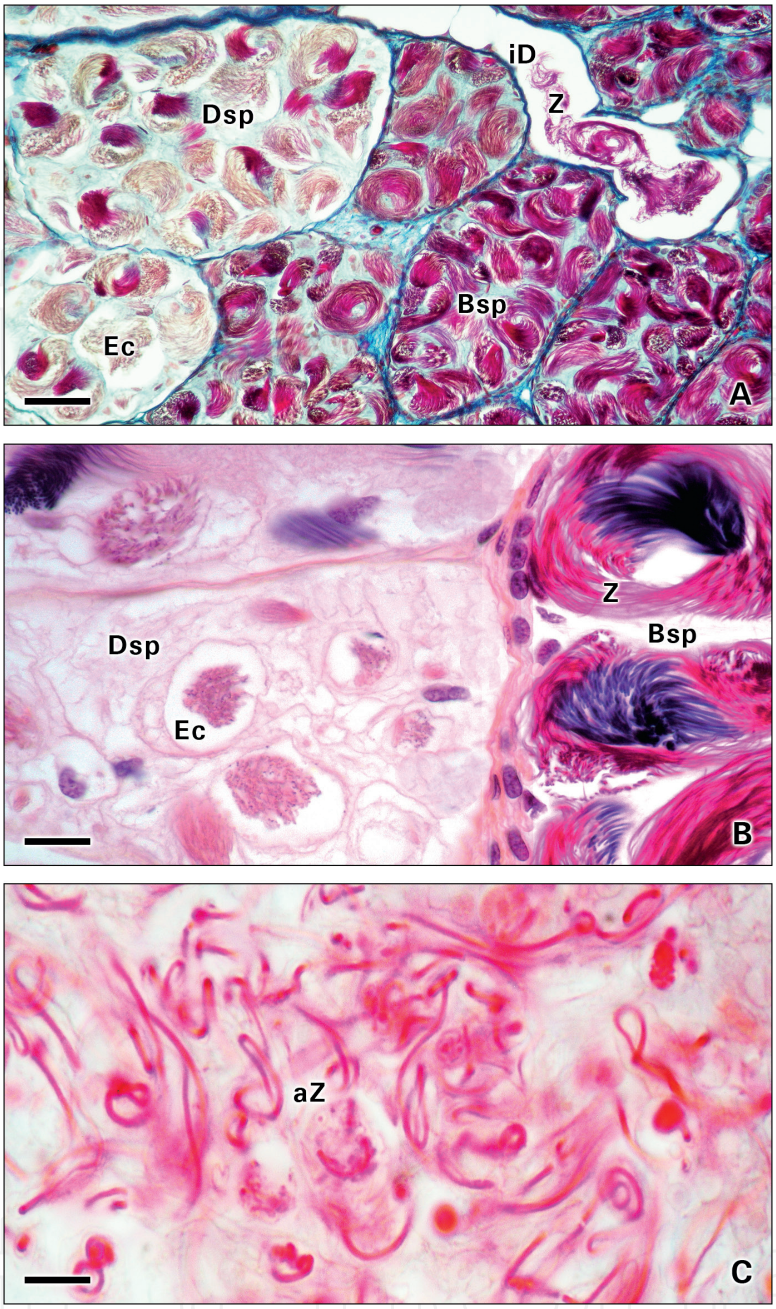

Figure 9. Spermiation in the testis of *Ambystoma dumerilii*. (A, B) Portion of the testis where is seen the limit between the regions before and during spermiation. The region before spermiation contains lobules with abundant cysts with spermatozoa. The region during spermiation contains few cysts with spermatozoa, compared with these seen in the other region and there are also empty cysts, without spermatozoa, containing only the residue of the Sertoli cells. A portion of an intratesticular duct containing spermatozoa is seen. Masson's trichrome. Bar = 0.1 mm. H-E. Bar = 20 μm. (C) During spermiation, abnormal spermatozoa, showing irregular morphology, may remain into the cysts. H-E. Bar = 20 μm. Testicular regions: Before spermiation (BSp) and during spermiation (DSp), spermatozoa (Z), empty cyst (Ec), intratesticular duct (iD), and abnormal spermatozoa (aZ).

Upon the conclusion of the spermiation, when the cysts open and the spermatozoa leave the testis, Sertoli cells remain inside the lobule and undergo morphological changes during their degeneration until they disappear [9, 19, 24]. During the emptying of the cysts, some spermatozoa remain in some of the cysts which show abnormal morphology (**Figure 9C**); these spermatozoa are phagocytized by the Sertoli cells [14].

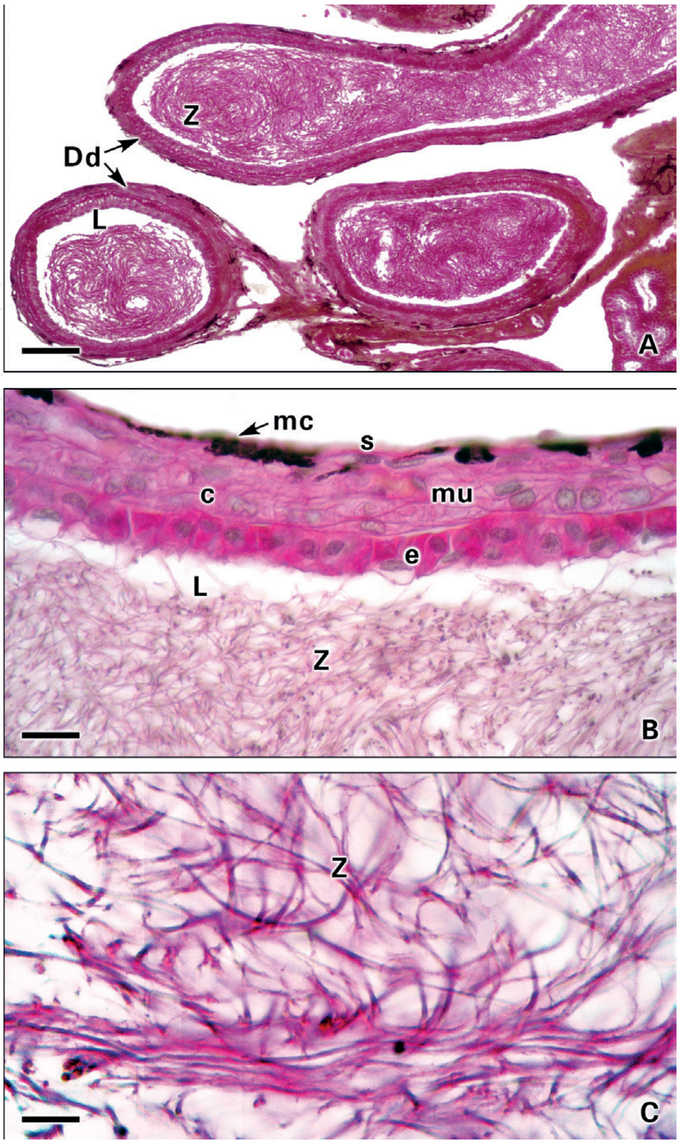

Figure 10. Deferent duct of *Ambystoma dumerilii*. (A) Sections of the large and folded deferent duct containing abundant spermatozoa in the lumen. B: H-E, C: PAS. Bar = 0.1 mm. (B) Detail of the deferent duct lined by cuboidal epithelium, connective tissue, muscle cells, and serosa. Subjacent the serosa, there are melanocytes. B: H-E, C: PAS. Bar = 20 μm. (C) Spermatozoa in the lumen of the deferent duct. B: H-E, C: PAS. Bar = 10 μm. Deferent duct (Dd), spermatozoa (Z), lumen (L), cuboidal epithelium (e), connective tissue (c), muscle cells (mu), serosa (s), and melanocytes (mc).

Throughout spermiation, spermatozoa are progressively released from the cysts to the lobular lumen and then to the efferent duct system [14, 16, 17, 19, 24–26].

Intratesticular ducts (*rete testis*) are embedded in the interlobular connective tissue of the testis (**Figure 9A**). Their lumen is lined with squamous epithelium. The efferent ducts include cephalic mesonephric nephrons, corresponding to the type of mesonephric kidneys of amphibians. The nephronic collecting ducts empty into the vas deferens also called primary urinary duct or Wolffian duct [2]. The Wolffian ducts are the largest of the sperm collecting ducts (**Figure 10A**); their lumen is lined with cuboidal epithelium and subjacent there are connective tissues, smooth muscle cells, and serosa; at the periphery, some melanocytes are dispersed (**Figure 10B** and **C**). In the lumen of the deferent ducts, the spermatozoa are in irregular position (**Figure 10C**); the cystic condition maintained all along the spermatogenesis is ended when the cyst is open at the spermiation, in the testicular lobules, before the entrance to the deferent duct system.

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