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ORCHESTRA FOR THE DRESS CODE OF SPERM AND ITS TRANSPORT IN CAECILIANS

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SUMMARY

Caecilians, of the order Gymnophiona in the Lissamphibia, are circumtropically distributed across most moist tropical regions of the world. Gymnophiona are among the least understood terrestrial vertebrates and have received lesser attention than the other two orders of lissamphibians, the Urodela and Anura. Spermiogenesis, known as spermateleosis in lower vertebrates, is the transformation of the round spermatid into a highly specialized spermatozoon with a species-specific structure. Spermateleosis and sperm morphology of two species of caecilians, *Ichthyophis tricolor* and *Uraeotyphlus cf. narayani*, from the Western Ghats of Kerala, India, were studied using light and scanning electron microscopy. The cyst morphology is described in ten steps. We also report the sperm transporting course in this enigmatic amphibian in addition to localizing sperm in the kidney. This confirms the transport of sperm through the kidney.

Key words: Ameboid cells; Caecilians; Connective tissue strand; Cyst; Sertoli cells; Spermatids; Spermatozoa; Sperm transport.

INTRODUCTION

Gymnophiona (caecilians) are the least well-known amphibians with intriguing morphological, physiological, reproductive, and behavioral traits. An understanding of the reproductive biology of caecilians may clarify several aspects of fossorial life, terrestrialization and viviparity in vertebrate evolution. Seshachar, during 1930s and 1940s using light microscopy, made outstanding contributions to the knowledge of spermatogenesis (1-4), spermateleosis (5, 6), sperm morphology (7) and sperm transport (1) in a few species of Indian caecilians. Later, Wake (8, 9) and Exbrayat (10), among the few, contributed further to our understanding of these in a few more caecilian species. However, several intricate features of male reproductive biology of this interesting group of animals have remained uninvestigated. Recently, we have described the Sertoli cells (11) and spermatogenesis (12) in *Ichthyophis tricolor* and *Uraeotyphlus cf. narayani* by combining light- and electron microscopy and identified stages in spermatogenesis comparable to those in the Anura and Urodela. However, information on spermateleosis in caecilians is available from only light microscopic observations by Seshachar (5, 6). Ultrastructural details of spermateleosis are described in three stages as early, mid and late phases by Smita *et al.* (13). The present paper describes the cyst morphology at the light microscopic level. The morphology of the cysts/locules and the organization of the spermatid are so varied that several clear distinguishable categories would be identified. Based on the distribution there on, we have made an attempt to allocate each category as a step in the process of spermiogenesis or spermateleosis i.e., transformation of the round spermatid into the sperm for spermiation.

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In the anurans and urodeles, sperm reach the kidney through the mesonephric duct and then take the same course, as urine, for ejaculation (14, 15). In caecilians, sperm from the testis lobules reach the collecting ductules. Beyond that little is known about their arrival into the urinogenital duct. The only reference in this regard is that of Wake (8) who did not find sperm in any part of the kidney. Our elaborate study on male reproductive biology of caecilians (11-13,16) employing light and scanning electron microscopy helped us to locate sperm in the kidney tubules in *I. tricolor.*

MATERIALS AND METHODS

I. tricolor were collected from subterranean habitat plantations and coconut grooves of Thiruvananthapuram district of Kerala (17). Lobes of testis, interlobular tissue, the mesentery connecting testis lobes and kidney, kidney and urinogenital duct were fixed in Bouin's fluid, embedded in paraffin wax and serial sections were stained in H & E for observation in Carl Zeiss Axiovision research microscope. Tissues washed in phosphate buffered saline were fixed in glutaraldehyde, dehydrated in ethanol, treated with isoamyl citrate, coated with gold palladium complex and examined with a Philips Excel 30 scanning electron microscope.

RESULTS

Morphology of the cyst variation to attain the characteristic dress code for sperm is described in ten steps. An attempt to trace the transport of sperm was also made.

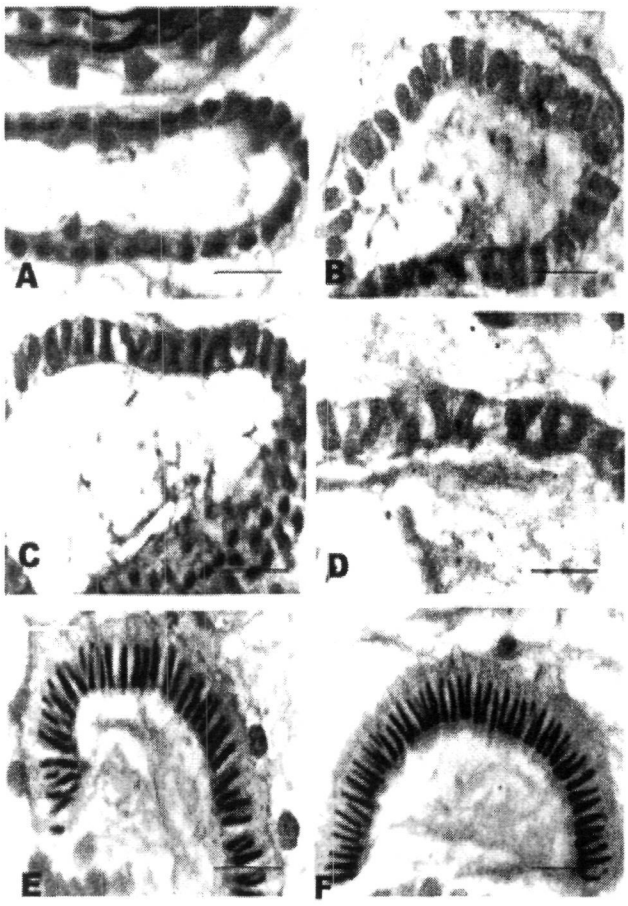

Fig. 1A: Section showing step I in spermiogenesis where spermatids elongate around the periphery of a central lumen. Scale bar = 30 μm

B: Step II spermiogenesis where cells become cylindrical and elongate transversely. Scale bar = 30 μm.

C: Step III spermiogenesis in which cells are interconnected and nucleus is strongly basophilic. Scale bar = 30 μm.

D: Step IV. Chromatin is clear and nucleus shows consolidation and pleomorphy. Scale bar = 30 μm.

E: Step V. Nucleus forms a compact cylindrical rod and ameboid cells lie on the cytoplasmic phase of spermatids. Scale bar = 30 μm.

F: Step VI. Cyst becomes arched and cytoplasm along the outer face of the cytoplasm contain flagella. Scale bar = 30μm.

Step I

Spermatids, which are small round cells with eosinophilic cytoplasm and a spherical basophilic nuclei, measure 10 μm in diameter (Fig. 1A). The cyst lies along the inner boundary and adherent to the Sertoli cells. The cells are organized in such a way as to surround a large lumen. All around the outer face, the germ cells remain adhered to the processes of Sertoli cells.

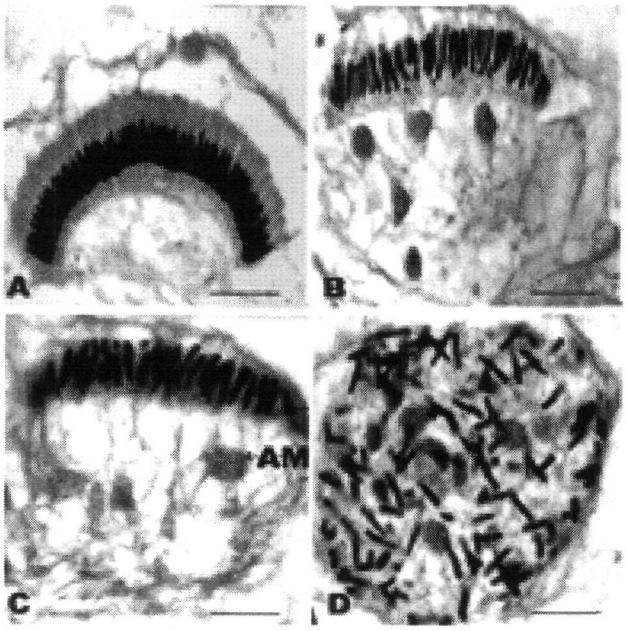

Fig.2 A: Step VII. Head of differentiating spermatid faces the lumen of the cyst and the flagellum away from the lumen. Scale bar = 30μm.

B: Step VIII. Shows the change in morphology of the arch. Scale bar = 30μm.

C: Step IX. Mature spermatozoa with distinct head and tail. Ameboid cells are present inside the lumen of the cyst. Scale bar = 30μm.

D: Step X. Sperm bundle and ameboid cells. Scale bar = 30μm.

Step II

Spermatid nuclei become cylindrical and elongated transversely at an angle tangential to the diameter of the cyst (Fig.1B). Broad nuclei have a length of 14 μm and a width of about 7 μm. The cell nests are attached to the lobule wall. The hyaline area between cells has increased, reflecting further development of the intercellular spaces. Sertoli cell cytoplasm is seen inside the lumen.

Step III

The cells are interconnected and further elongated transversely (Fig. 1C). The nucleus is strongly basophilic and at the same time the cells become more compact through appearance of prominent intercellular spaces. Scanty filamentous cytoplasm is present. The nucleus measures about 12 μm in diameter in the longest axis and 5 μm across and lumen width is about 80 μm.

Step IV

The chromatin is more clear and the nucleus occasionally develops a pleomorphic morphology (Fig. 1D). Consolidation of the spermatid nucleus occurs. The end away from the lumen shows the formation of tail. Nucleus measures about 17 μm in the longest axis and 5 μm across.

Step V

There is shrinkage and condensation of the nucleus resulting in the formation of compact cylindrical spermatid. The pinkish nucleus is changed to dark blue elongated nucleus, with a nuclear

diameter of 15 μm in the long axis and 2 μm across. Corresponding to the condensation and elongation of the spermatids, the overall size of the cyst/locule decreases. The spermatid heads are arranged in a manner as an arch on top of a central lumen with the ameboid cells lying in the cytoplasmic phase of those spermatids (Fig.1E). Sertoli cell cytoplasm is seen in the inner face of the cyst.

Step VI

The cyst is arched and the spermatid becomes further elongated (Fig. 1F). The nucleus is further condensed and compact, and measures about 15 μm long and 2 μm wide with a lumen height of 55 μm and a diameter 100 μm. Ameboid cells are prominent. The width of the arch is decreased. Outer face of the arch contains flagella. Acrosome is embedded in the Sertoli cell cytoplasm.

Step VII

During this step, the arch has further decreased in width (Fig. 2A). The cells have become more compactly arranged with little, if any, intercellular space. The head of the differentiating spermatid faces the lumen of the cyst and the cytoplasm is richly eosinophilic. The nucleus measures about 15 μm in the long axis. Lumen length is 80 μm and width 30 μm. Ameboid cells are still prominent.

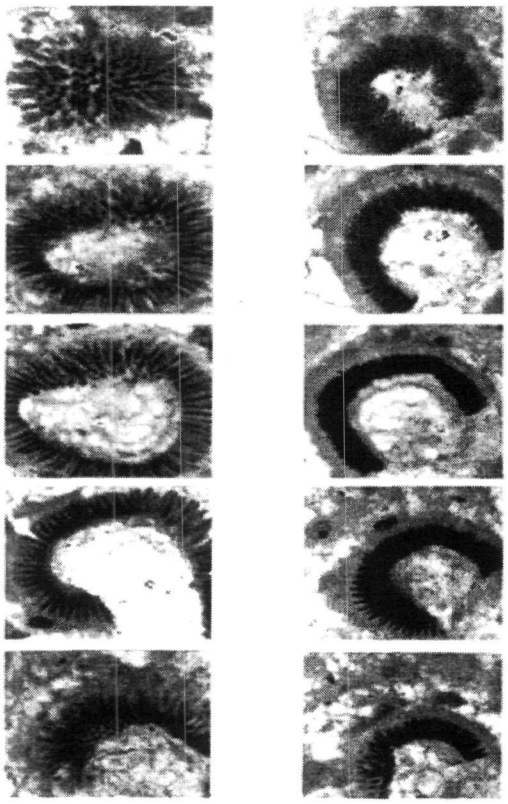

Fig.3. Serial sections to reflect the three - dimensional organization of cysts.

The cyst further decreases in size, and the spermatid arch has become almost flat (Fig. 2B). Spermatid nuclei measure a length of about 15 μm and width of 2 μm. There is less eosinophilic cytoplasm in the outer as well as in the inner aspects of the cyst wall. Ameboid cells have increased in abundance and are found among the flagella. They may probably be involved in the pinching off and phagocytosis of the residual cytoplasm. The Sertoli cell cytoplasmic extension is still clear.

Step IX

The spermatids are now changed into mature spermatozoa. The spermatozoon of *I. tricolor* is filiform with a darkly stained compact cylindrical rod-like nucleus (Fig. 2C). Head and tail are distinct. The nucleus measures about 13 μm in length. Ameboid cells of about 10μm are present among flagella.

Step X

The spermatozoa with pointed head are fully differentiated. The stronger basophilia are towards the flagellum and acidophilia towards the pointed end reflecting the acrosome. The sperm disaggregate from the arch and lie free in the lobule. Ameboid cells lie in admixture with the sperm (Fig. 2D).

Three dimensional organization of spermatid cysts

An attempt was made to analyse the organisation of the spermatid cysts at steps VI and VII to have an impression about the three dimensional organisation of the respective cysts (Fig.3). The testis lobes (10-11) on each side are connected by a connective tissue strand (CT) and not by a longitudinal duct as described earlier (1). The circular profile of the CT strand is such that in a longitudinal section it appears to be a duct for a casual observer (Fig. 4A, B). Sperm produced in the testis lobules arrive at collecting ductule which traverse through the interlobular tissue, join among themselves and leave the lobule to continue beyond it. Those ductules which take a straight transverse course reach the kidney. The others which take a vertical course reach the interlobular CT strand and then take a transverse course toward the kidney. This is clearly evident in our histological preparations (Fig. 4C). Probably, Seshachar (I) considered the interlobular CT strand as a longitudinal sperm ducts. We have definite histological evidence for sperm localization in the collecting ducts of the peripheral nephrons in the kidney (Fig. 4D).

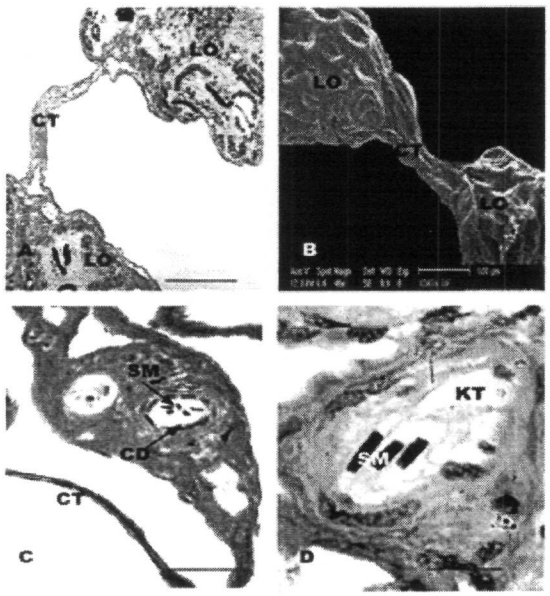

Fig.4 A: *Ichthyophis tricolor*. Testis lobes (LO) are connected by a connective tissue strand (CT). H & E stained paraffin section. Scale bar = 40 μm.

B : *Ichthyophis tricolor*. Testis lobes (LO) are connected by a connective tissue strand (CT). SEM. Scale bar = 50μm.

C : *Ichthyophis tricolor.* Presence of collecting ducts (CD) with sperm (SM) in the connective tissue strand (CT). H & E stained paraffin section. Scale bar = 20μm.

D : *Ichthyophis tricolor.* Sperm (SM) inside the kidney tubule (K). H & E stained paraffin section. Scale bar = 20 μm.

DISCUSSION

Spermateleosis in caecilians had been described using light microscope by Seshachar (5, 6), Exbrayat and Sentis (18) and Exbrayat (19, 20). Spermiogenesis is an extraordinarily complex process involving elaboration of the acrosome from the Golgi apparatus, condensation and elongation of the nucleus, formation of a motile flagellum and shedding of the excess cytoplasm. Though the steps in detail are described only for *Ichthyophis tricolor*, it may well be applied to the other species also. At the beginning of spermiogenesis, the spermatids are round globular cells with eosinophilic cytoplasm and spherical basophilic nuclei. The cells begin to elongate around a large central lumen with a nuclear diameter of 10 μm and lumen width of 35 μm. Gradually, the elongation continues. In mammals, the nuclei of young spermatids, after Fuelgen staining, show several karyosomes dispersed in a fine dusty chromatin. The nucleus of the early spermatids and that of fully formed spermatozoa are always very different in size, shape and form; the former is generally spherical whereas the latter exhibits a great variety of forms.

It has been reported by Seshachar (21) that one important change in spermateleosis from spermatid to the adult sperm is one of the consolidation and condensation of the nucleus. The consolidation implies a reduction in the nuclear volume. Though Seshachar (6, 21) described spermateleosis depicting the changes in the acrosome, centriole, nucleus and mitochondria, the cyst morphology was not explained in his study. We have described the orchestra for the change in morphology to attain the characteristic shape of sperm in ten steps. In the light microscopic observations of Seshachar (6, 7) the sperm of *Ichthyophis glutinosus, Ureotyphlus narayani, Siphonops annulatus,* and *Gegeneophis carnosus* are spatulate. We report a pointed head for *Ichthyophis tricolor.* As reported in earlier descriptions (22-25), the nucleus in the mature sperm resembles a relatively short cylinder of constant diameter, circular in cross section, with strongly condensed chromatin. We also report a significant link hitherto not shown in the course taken by sperm from the testis lobule to the urinogenital duct, i.e., through the kidney in a caecilian. It also clarifies that there is no longitudinal sperm duct i.e., in caecilians but only connective tissue strand with a circular profile connecting the testis lobules. Vasa efferentia take two different courses, 1). straight from the testis lobules to the kidney and 2) a deviated route of interlobular CT strand. We suggest that sperm taking a devious route through the interlobular CT strand is a device to store them for short to long periods as an adaptation of seasonal reproduction.

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REFERENCES

1 Seshachar BR (1936). The spermatogenesis of *Ichthyophis glutinosus* L. The spermatogonia and their division. *Z Zellforsch mikr Anat Bd* **24:** 662-706.

2 Seshachar BR (1937). The spermatogenesis of *Ichthyophis glutinosus* (Linn.) II. The meiotic divisions. *Z Zellforsch Mikr Anat* **27:** 133-158.

3 Seshachar BR (1939). The spermatogenesis of *Uraeotyphlus narayani*. *La Cellulae* **48:** 63-76.

4 Seshachar BR (1942). Stages in the spermatogenesis of *Ichthyophis glutinosus* Mikan. and *Schistometopum gregori* (Blgr) (Amphibia: Apoda). *Proc Ind Acad Sci* **15:** 263-277.

5 Seshachar BR (1943). Spermatogenesis of *Ichthyophis glutinosus* Lin. III. Spermateleosis. *Proc Natl Inst Sci India* **9:**271-286.

6 Seshachar BR (1945). Spermateleosis in *Uraeotyphlus narayani Sesh.* and *Gegeneophis carnosus* Bedd. *Proc Natl Inst Sci India* **11:** 336-340.

7 Seshachar BR (1940). The apodan sperm. *Curr Sci* **11:** 439-441.

8 Wake MH (1968). Evolutionary morphology of the caecilian urogenital system. Part. The gonads and fat bodies. *J Morphol* **126:** 291-332.

9 Wake MH (1977). The reproductive biology of caecilians: An evolutionary perspective. In: Taylor DH and Guttman SI (eds), *The Reproductive Biology of Amphibians*. Plenum Press, New York. pp. 73-101.

10 Exbrayat JM (2000). *Les Gymnophiones, ces curieux Amphibiens*, Editions Boubée, Paris, p 443.

11 Smita M, Oommen OV, Jancy MG and Akbarsha MA (2003). Sertoli cells in the testis of caecilians, *Ichthyophis tricolor* and *Uraeotyphlus cf. narayani* (Amphibia: Gymnophiona): Light and electron microscopic perspective. *J Morphol* **258:** 317-326.

12 Smita, M, Oommen, OV, Jancy, MG, Akbarsha, MA (2004). Stages in spermatogenesis of two species of caecilians *Ichthyophis tricolor* and *Uraeotyphlus cf. narayani* (Amphibia: Gymnophiona): Light and electron microscopic study. *J Morphol* **261:** 92-104.

13 Smita M, Jancy MG, Girija R, Akbarsha MA and Oommen OV (2004). Spermiogenesis in caecilians *Ichthyophis tricolor* and *Uraeotyphlus cf. narayani* (Amphibia: Gymnophiona): Analysis by light and transmission electron microscopy.

14 Saidapur SK (1989). Reproductive cycles of Amphibians. In: Saidapur SK (ed.), *Reproductive cycles of Indian Vertebrates*, Ahmedabad. India. Allied Publishers Ltd, pp.166-188.

15 Lofts B (1974). Reproduction. In: Lofts B (ed.), *Physiology of the Amphibia*. Academic Press, New York, pp107-218.

16 George JM, Smita M, Oommen OV and Akbarsha MA (2004). Histology and ultrastructure of male mullerian gland of *Uraeotyphlus narayani* (Amphibia: Gymnophiona). *J Morphol* **260:** 33-56.

17 Oommen OV, Measey GJ, Gower DJ and Wilkinson M (2000). Distribution and abundance of the caecilian *Gegeneophis ramaswamii* (Amphibia:Gymnophiona) in Southern Kerala. *Curr Sci* **79:** 1386-1389.

18 Exbrayat JM and Sentis P (1982). Homogénéité du testicule et cycle annual chez *Typhlonectes compressicaudus* (Duméril et Bibron, 1841) Amphibien Apode vivipare. *C R Acad Sci Paris* **292:** 417-420.

19 Exbrayat JM (1986). Le testicule de *Typhlonectes compressicaudus*: structure, ultrastructure, croissance et cycle de reproduction. *Mém Soc Zool Fr* **43:** 121-132.

20 Exbrayat JM (1986). Quelques aspects de la biologie de la reproduction chez *Typhlonectes compressicaudus* (Duméril et Bibron, 1841). Résumé de thèse. *Bull Soc Herpét Fr* **38:** 19-22.

21 Seshachar BR (1943). Volume relations of the nucleus in apodan spermateleosis. *Proc Ind Acad Sci* **17:** 138.

22 Van der Horst G and van der Merwe L (1991). Late spermatid-sperm/ Sertoli cell association in the caecilian, *Typhlonectes natans* (Amphibia: Gymnophiona). *Elect Microsc Soc S Africa* **21:** 247-248.

23 Van der Horst and G van der Merwe L (1991). The ultrastructure of the spermatozoon of *Typhlonectes natans* (Gymnophiona: Typhlonectidae). *J Herpetol* **25:** 441-447.

24 Jamieson BGM (1999). Spermatozoal phylogeny of the vertebrata. In: Claude Gagnon (ed.), *The Male Gamete. From Basic Science to Clinical Applications.* Cache River Press, USA, pp. 304-331.

25 Scheltinga DM, Wilkinson M, Jamieson BGM and Oommen OV (2003). Ultrastructure of the mature spermatozoa of caecilians (Amphibia: Gymnophiona). *J Morphol* **258:** 179-192.