From the Sertolian syncytium to Sertoli cells in anamniotes, especially Gymnophionan (Caecilian) Amphibians

J.-M. Exbrayat
Université de Lyon, UMRS 449, Laboratoire de Biologie Générale, Université Catholique de Lyon; Laboratoire de Reproduction et Développement Comparé, EPHE/PSL; 10, place des Archives, 69288 Lyon Cedex 02, France

In Vertebrates, seminiferous tubes are bordered with an epithelium producing gametes included between Sertoli cells. These cells were first described like a syncytium. With electron microscopy, the observations showed Sertoli cells contained a single nucleus and were limited by a classical plasma membrane. Some studies showed the Sertoli cells of anamniotes originated from follicle cells surrounding the cysts of germ cells. At the end of 1960’s the paradigm of Sertolian syncytium was out. But Sertolian syncytium remained described during a long time. In anamniotes, Sertoli cells constitute the wall of cysts grouping germ cells. In burrowing or aquatic lengthened gymnophionan (caecilian) amphibians, the testes are constituted with several lobes containing locules in which spermatogenesis occurs. Light microscopy indicated gametes were grouped in cysts included into a filamentous matrix containing fat globules. Some nuclei were observed in a peripheral position. For a long time it was considered the Sertoli cells of these animals were limited to those nuclei with a very small cytoplasm. The use of SEM and TEM revealed the matrix belonged to the cytoplasm of Sertoli cells. The fibrous aspect corresponded to an artefact due to the fixative. The study of the testis development showed Sertoli cells were giant cells delimited with a plasma membrane. Finally, in these amphibians, the structure of Sertoli cells is reminiscent of the Sertoli cells belonging to other anamniotes.

Keywords: Sertoli cell, testis, germ cell, anamniote, amphibian, gymnophionan

1. Introduction

In Vertebrates, testes are constituted with seminiferous tubes or ampullae bordered with a germinal epithelium producing gametes. This epithelium is put on a connective wall by a basal lamina. Endocrine Leydig cells are included into connective tissue. Germinal epithelium is constituted with cells of spermatic lineage: spermatogonia, spermatocytes I and II, spermatids and spermatozoa. This epithelium also contains somatic Sertoli cells between which are included germ cells. This pattern is found in amniotes and anamniotes, with some differences in their organization. Firstly studied in man and mammals, Sertoli cells were considered as merged, giving a syncytium. The use of TEM allowed one to find Sertoli cells were well individualized, but a long time has been necessary to accept this new view of Sertoli cells.

2. A brief story of the discovery of Sertoli cell

2.1 The discovery of Sertoli cells: syncytium or single cells?

Genital tracts were subject of interrogation since a long time. After several centuries of macroscopical observations and deductions often imprinted with philosophy, the invention of microscope by Hooke and Van Leeuwenhoek allowed to increase the knowledge of these organs. Van Leeuwenhoek (1632-1723) was one of the first to describe spermatozoa (*animalcule spermatici*) in 1677. Later, Spallanzani in 1780, performing a famous experiment in frog, showed the seminal fluid was at the onset of fertilization of female oocytes. XIXth was to be expected to meet several researchers working on male gonads. Among these researchers, Sertoli published a first description of “branched cells” in human testes [1]. These branched cells constituted the Sertolian syncytium. He also published fundamental observations on spermatogenesis, resuming Von Ebner’s hypothesis [2]: the spermatozoa were issued from the branched cells. Sertoli thought these cells performed a nutritional or mechanical role in spermatogenesis. He also showed spermatozoa were coming from rounded spermatids (called nematoblasts), he described spermiogenesis dividing the development of spermatocytes into three steps: leptotene/zygotene, pachytene, and diplotene. Spermatogonia were divided into two stages. Sertoli observed some waves of spermatogenesis in which germ cells evolved grouped into isogenic series, and linked each other with cytoplasm bridges.

2.2 The persistence of the “syncytium paradigm”

Sertoli cells were described in 1865 such as branched, i.e. like a syncytium, a cell mass with a lot of nuclei. But Sertoli using only light microscopy, it was not possible for him to visualize the plasma membrane of these cells with this technique. So, all nuclei appeared to be included into the same cytoplasm. Even Sertoli already suggested the branched cells were a mass of single cells from which the limits could not be observed [3], the syncytium concept was integrated into the scientific community, exceeding Sertoli himself. Regaud [4,5] was opposed to Sertoli’s deduction of cells with
a single nucleus [6], and a lot of scientists adopted the “Sertoli syncytium” (Fig. 1). In the necrology of Pol Bouin (1870-1962), Robert Courrier [7] spoke about the “Sertoli syncytium” studied by Pol Bouin and Paul Ancel which gave about twenty publications in 1903 and 1904. In reports about the castration of foals performed by Bouin and Ancel, the authors always spoke of a syncytium which was unique in seminiferous tubule; in cryptorchid pigs, they noted the absence of Sertoli syncytium. Gopalakrishna studying the embryology of chiropterans noted: “the Sertoli cells are not so distinctly marked out. Their nuclei are small and more densely stained, and they appear to be floating in the Sertoli syncytium.” [8]

During several years, “Sertoli syncytium” was always used but some authors were lesser precise than others. Allen explained the “indifferent cells (another name given to Sertoli cells) had very slight if any visible membranes” [9]. Aas-Jorgensen spoke about the scarcity of cells with several nuclei in the testis [10]. Yet, some authors at the beginning of XXth considered Sertoli cells being well-delimited units, like most other cells [11, 12], others considered the cell membranes were destroyed by fixative [13].

Observed with light microscope, the limits of Sertoli cells were not visible or very little visible, the cytoplasm was clear, nucleus oval with a big nucleolus and a characteristic indentation which permitted to recognize them among the germ cells. They seemed to be never mitotic in adult. When TEM generalized in biology, the observations showed Sertoli cells were limited with a classical plasma membrane and contained a single nucleus [14,15], even if sometimes it was not possible to visualize these limits with electron microscope [16]. Fawcett and Burgos rejected the concept of germ cells included into Sertoli cells for they observed plasma membranes separating the two kinds of cells [17] (Fig. 2). Sapsford et al. did not visualize the limits of Sertoli cells but they dissociated Sertoli cells from seminiferous tubules, showing they were cells with a single nucleus [6, 18, 19]. From the time at which the true structure of Sertoli cell has been established, a lot of studies using electron microscopy were published, permitting to understand the relationships between Sertoli and germ cells. Some studies also showed the Sertoli cells of anamniotes originated from follicle cells surrounding the cysts of germ cells [20]. Finally, at the end of 1960’s one could think the paradigm of Sertoli syncytium was out. But it was not exact.

Fig. 1: Schematic representation of “Sertoli syncytium”. The lumen of seminiferous tubule is located on the upper part of picture. Basal lamina on which the syncytium is put is located on the lower part. Germ cells are included into the syncytium. 1: nuclei of Sertolian syncytium; 2: germ cells (oocytes II); 3: spermatozoa ready to be released in the lumen; 4: Sertoli syncytium.

Fig. 2: Schematic representation of Sertoli cells in amniotes. The lumen of seminiferous tubule is located on the upper part of picture. Basal lamina on which the Sertoli cells are put is located on the lower part. Germ cells are included between Sertoli cells. 1 and 1’: nuclei of two cells; 2: germ cells (oocytes II); 3: spermatozoa ready to be released in the lumen; 4: Sertoli cell.
2.3 From syncytium to single cell

In several books for teaching, Sertoli syncytium was described during a long time. In 1967, Houillon spoke about “Sertoli syncytium” [21], but he also specified the limits of cells were hard to see. In 1973, Gallien also spoke about a Sertoli syncytium highlighted into ductules without any sperm cell in the cases of free-martin [22]. Boue and Chanton explained Sertoli cells anastomosing to form a syncytium [23]. It is easy to imagine a more or less long time (2 to 3 years) can pass without any update between the redaction of a book and its publication. But, curiously the Sertoli syncytium was found in several more recent articles: some authors [24,25] explained the germ cells of the snake *Tantilla hobartsmith* were packed into a Sertoli syncytium. A comparable situation was described in the snake *Seminatrix pygmaea* [26].

3. Sertoli cells in anamniotes

3.1 Sertoli cells in Agnathans, Chondrychtyans, and Osteichyans

The first studies about Sertoli cells concerned man, then mammals and other amniotes (reptiles and birds), and currently, a lot of studies concerning mammals were published [20]. Few works were devoted to anamniotes’ Sertoli cells. In anamniotes of which sexual cycles are frequently discontinuous, successive generations of germ cells may be found at each spermatogenic wave [27].

In Agnathans, somatic cells were described in testes [28]. They were called “vegetative cells” [29], “link cells” or “Sertoli cells” [30]. In Osteichyans, the organization of seminiferous tubules was described in several species [31]. They were described in *Latimeria chalumnae* [32], *Protopterus sp.* [33]. In Teleosteans, seminiferous tubules or locules are limited with a layer of somatic cells sometimes called “Sertoli cells”, “follicle cells”, or “connective cells”. For other authors, cells looking like amniote’s Sertoli cells were called “lobules boundary cells” [34,35]. Histochemical investigation showed these cells possessed a steroidogen glandular function, and some authors considered them to be the equivalent to Leydig cells [34, 36-44]. Henderson also spoke about “link cells” but without attributing them any endocrinial function [45]. Some authors considered Sertoli cells or link cells such as a syncytium [37,38].

In the abundant literature of the three first thirds of XXth, the terminology of Sertoli cells or link cells in Osteichyans was well variable, and a steroidogen function could or not be attributed to these cells. Studies from 1960 showed these Sertoli cells able to synthesize steroid hormones. So some authors proposed to speak about Sertoli cells in all the cases [31]. Using TEM, these authors studied them in species performing a yearly reproductive cycle in order to observe testes filled with all the stages of germ cell or, contrarily, containing only spermatogonia. These observations gave well contrasted situations of the same structure throughout time. The species studied were *Salmo trutta* and *S. gairdneri* in which follicle cells or link cells were described, *Esox lucius* with link cells, *Cyprinus carpio* with Sertoli cells, and *Rutilus rutilus* with lobules boundary cells or Sertoli cells. During quiescence, Sertoli cells surrounded isolated or pair-grouped spermatogonia [31]. At spermatogenesis, each spermatogonium divided, daughter cells being separated each other with Sertoli cells. Each spermatogonium originated a cyst in which divisions were synchronous (isogenic series). Into each cyst, spermatogenesis was independent from the other cysts. At the end, when spermatooza were formed, the cyst wall constituted with the well thin cytoplasm of Sertoli cell, opened and spermatooza evacuated into the tubule. Sertoli cells became now against the wall of seminiferous tubule. Some of them lost fragments of cytoplasm at the spermatooza evacuation.

The first studies about Sertoli cells In Chondrichytans were published by Swaen and Masquelin [46], and Stephan [47]. TEM permitted to precise the structure and ultrastructure of Sertoli cells in Selachians throughout spermatogenesis [48,49]. Like in Osteichyans, the Chondrychtyan seminiferous tubules were constituted with several cysts still called ampullae in which spermatogenesis occurred synchronously in each one. These cysts originated from the spermatogonia situated in a ventral position into the testis of *Scyllorhinus canicula* [48]. Throughout their evolution, these cysts constituted with isogenic series of germ cells, migrated in a dorsal position and finished to empty mature spermatooza in efferent ductules. The empty cysts degenerated. The differentiation of a seminiferous tubule began with the association of a Sertoli cell and a spermatogonium. In Selachians, Sertoli cells contained “problematic bodies” [50] corresponding to proteins involved in the epithelium lysis when spermatooza became free. Several successive divisions of both the two cell types drove to the increase of seminiferous tubule. Each Sertoli cell was then associated to several germ cells divided in a synchronous manner, giving isogenic series. Several cysts were observed with different states of spermatogenic differentiation into the same seminiferous tubule. At meiosis, Sertoli cells increased. In *Squalus acanthis*, when spermatooza became mature, they were thrown into the lumen after degeneration of all or part of Sertoli cell in which spermatooza were included, with the presence of “problematic bodies” [48].

3.2 Sertoli cells of anuran and urodelan amphibians

In amphibians, some authors published literature showing an organization looking like that of other anamniotes [51,52] (Fig. 3). During spermatogenetic activity, the spermatooza issued from the proliferation of germ cells grouped into homogenous cysts limited with follicle cells (these follicle cells were in fact the first stage of Sertoli cells). In Anura,
the cells surrounding the cyst increased, becoming steroidogenous. They separated from the germ cells having reached the spermatozoa stage by the aperture of the cyst. Follicle cells now free from germ cells became Sertoli cells and looked like amniotes’ ones. A study with TEM performed in the toad *Bufo arenarum* showed these Sertoli cells were well limited by a plasma membrane and were not grouped such as a syncytium [53]. These authors also noted the shape modification of these cells from the time at which they were follicle cells surrounding the isogenic series of germ cells, and the time at which the cysts contained spermatids or spermatozoa. At this time, the structure of amphibian Sertoli cells looked like anamniote’s ones even if Lofts insisted on the difference between anamniote and amniote Sertoli cells [51,52].

**Fig. 3:** Schematic representation of Sertoli cells in anamniotes. The lumen of seminiferous tubule is located on the upper part of picture. Basal lamina on which the syncytium is put is located on the lower part. Germ cells are included between Sertoli cells, grouped in order to form cysts which are clusters grouping cells at the same stage of differentiation. 1: oogonium; 2: germ cell (oocytes II); 3: nucleus of Sertoli cell; 4: Sertoli cell; 5: germ cell (oocyte I ready to be released in the lumen; 6: spermatozoa ready to be released in the lumen; 7: Sertoli cell (follicle cell) opened to release spermatozoa.

3.3 Sertoli cells in gymnophiona (Caecilians)

Gymnophionan amphibians have a special place among the anamniotes [54]. These lengthened and burrowing animals live on tropical areas. They possess several specific characteristics. The testes are constituted with several lobes which can be more or less fused. Each lobe contains several locules, in which spermatogenesis occurs. Each locule is linked by a duct to the rete testis in which spermatozoa are driven to the cloaca using the Wolffian ducts. The locules are separated each other with connective tissue containing steroidogenic Leydig cells. The disposal of germ cells is specific to Gymnophiona. The primary spermatogonia are situated at the onset of evacuative duct, secondary spermatogonia are situated against the wall of locule, and spermatocytes I and II, spermatids and spermatozoa differentiate in displacing toward the centre of locule. With light microscopy, cells belonging to the same stage are grouped in cysts seeming included into a filamentous matrix containing fat globules. Nuclei looking like the nuclei of Sertoli cells in the other vertebrates were observed in a peripheral position (Fig.4). This description is that which has been given since along time [55-58]. Seshachar considered these Sertoli cells were limited to nuclei contrarily to that it was known in other vertebrates (in which it was still a syncytium) [59]. Then no work about these cells in Gymnophiona was published during a long time.

**Fig. 4:** Transversal section of a lobe of *Typhlonectes compressicauda* testis. Staining: Masson-Goldner trichroma. Each lobe is divided into several locules. Each locule is filled with a gelatinous and filamentous matrix which corresponds to the cytoplasm of Sertoli cells. Germ cells seem to be included into this matrix, but they are grouped into clusters which are really included between Sertoli cells. ed: evacuating duct; gc: germ cells (here, spermatocytes) included into a cyst; loc: locule; m: matrix (cytoplasm of Sertoli cells); IT: interstitial tissue (Leydig cells); z: spermatozoa ready to be expelled. Scale bar = 30µm.

In 1986, Exbrayat resumed the study of Caecilian testis using particularly SEM and TEM [60]. These methods helped to understand the matrix was in fact the cytoplasm of Sertoli cells (Fig. 5). The fibrous aspect observed with
light microscopy corresponded to an artefact linked to the fixative. With SEM, these filamentous structures among them nuclei were scattered corresponded to the collapsus of cytoplasm structures. With TEM, it was obvious that Sertoli cells were particularly large. In the cytoplasm vesicles seemed empty but they were certainly filled with lipids before the operation of fixation and preservation into ethanol. The study of the testis development in embryos and young animals showed the evolvement of Sertoli cells showing it was well giant cells, delimited with plasma membranes [60-63] (Fig. 6). Finally, a lot of fat vesicles are included into the cytoplasm of these Sertoli cells, giving a general aspect optically empty; nuclei included in this cytoplasm look like separated units which can be well observed with both TEM and SEM (Fig. 7). This new vision of the structure of gymnophionan Sertoli cells has been published for the first time in 1994 [64]. Since this time, the structure of locules and Sertoli cells in Gymnophiona were completed [65-68]. Finally, in these amphibians, the structure of Sertoli cells is reminiscent of the Sertoli cells belonging to other anamniotes [52,69].

Fig. 5  *Typhlonectes compressicauda* testis. Left picture: SEM observation of a lobe of *Typhlonectes compressicauda* testis. Germ cells are included into cysts dispersed between Sertoli cells the nucleus of which being found against the peripheral wall of locule. Scale bar = 30 μm. Right picture: TEM observation of a locule of *Typhlonectes compressicauda* testis. Germ cells (here, spermatocytes) are included between the Sertoli cells. The cytoplasm of these ones is filled with substances looking like a matrix. Scale bar = 3 μm. CS: peripheral nucleus of Sertoli cell; C1: 1st spermatocyte; G1: 1st spermatogonium observed against the peripheral wall of locule; m: matrix (cytoplasm of Sertoli cell; S: cytoplasm of Sertoli cell (also called “matrix”).

Fig. 6  *Typhlonectes compressicauda* testis. Left picture: transversal section of testis belonging to *Typhlonectes compressicauda* new-born. Locules are small, separated with interstitial tissue. Germ cells (oogonia) are situated near a small evacuating duct. Small Sertoli cells develop with a small matrix (cytoplasm). Scale bar = 8 μm. Right picture: transversal section of testis belonging to *Typhlonectes compressicauda* lesser than one year-old. Sertoli cells develop with a more abundant matrix (cytoplasm). Germ cells remain against the wall of testis, and they become farer from the evacuating duct. Scale bar = 8 μm. ED: evacuating duct; GC: germ cell (oogonium); IT: interstitial tissue; M: matrix.
Fig. 7 Typhlonectes compressicauda Sertoli cells. Left picture: a nucleus of Sertoli cell observed with TEM, with characteristic indentations. Scale bar = 1.5 µm. Right picture: a part of a Sertoli cell containing the nucleus observed with SEM. Scale bar = 1 µm

4. Conclusions

In the first descriptions, with the use of light microscopes exclusively, Sertoli cells were considered such as a syncytium filling all the seminiferous tubules and in which germ cells were included. Evolution of light microscopy did not allow going further in the appreciation of the structure of these cells for plasma membrane never has been highlighted. This was perhaps a fixative effect but also due to the complexity of the imbrication between Sertoli and germ cells.

In the 1950s and 1960s the use of TEM allowed to precise the exact nature of these cells showing the plasma membranes. The first works about these cells were performed in amniotes. Other studies also showed such cells in anamniotes in which a constant was observed: Sertoli cells first surrounded a spermatogonium which will give an isogenic series of germ cells, constituting a cyst which stays enveloped into the Sertoli cell. At the end of spermatogenesis, these Sertoli cells modify to give cells looking that of amniotes. Curiously, the syncytium notion persisted several years after the discovery of the plasma membrane and, still to-day, several papers give reference to Sertoli syncytium.

After these morphological works, others studies allowed to understand the activity of Sertoli cells [20], sustaining as well as nutritive cells, with a phagocytic activity, ridding the seminiferous tubules of degenerated germ cells, which is manifested by the accumulation of lipids. Sertoli cells are the target of FSH and testosterone, they secrete the anti-Müllerian hormone at the origin of the regression of Müllerian duct in males, excepted in some anamniotes such as anuran amphibians the males of which keeping sometimes vestigial Müllerian duct, and mostly Gymnophionan in which Müllerian ducts persist in males with a gland function participating to the sperm composition with an activity comparable to that of the prostate in mammals [70,71].

Acknowledgements Author thanks Catholic University of Lyon (UCLy) and Ecole Pratique des Hautes Etudes (EPHE) that allowed the freedom in organizing his research field and supported his work. He also thanks colleagues and students who participated since more than 35 years, to the studies on Gymnophionans.

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