Sertoli cell ultrastructure. I. A comparative study in immature, pubescent, adult and cryptorchid pigs

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Summary. Sertoli cells of immature pig appeared as a monomorphic cell population. Their cytoplasm contained abundant smooth ER and numerous ribosomes. Sertoli cell junctions began to appear at 6 weeks. In mature testicular tubules two types of Sertoli cells have been identified: typical Sertoli cells (A) with a light nucleus, and other cells (B) with a dark nucleus. A-cells extended from the basement membrane of the tubule to the lumen and showed the same fine structure as in other mammalian species (numerous filaments and ribosomes, abundant smooth ER, parallel-oriented microtubules, Charcot-Böttcher's crystalloids and typical junctions between adjacent Sertoli cells and with germ cells). B-cells had a limited cytoplasm containing smooth ER and numerous filaments. The two types of Sertoli cells were easily characterized by the presence of a large lipid droplet. In cryptorchid testis there were several types of Sertoli cells. The light ones were poor in organelles; the others resembled typical mature Sertoli A-cells but contained many microtubules and Charcot-Böttcher's crystalloids which formed large complexes. Well-characterized B-cells were not found but some cells similar to them were noted.

Introduction.

For a long time the Sertoli cell was considered as only having nutritive (Elftman, 1963; Fouquet, 1968) and phagocytic (Clegg and Mac Millan, 1965; Reddy and Sobo- bada, 1967; Carr et al., 1968; Sapsford et al., 1969) functions. Recently, other activities have been observed indicating that these cells play a key role in male sexual functions, i.e., establishment of a blood-testis barrier due to specialized junction systems (Dym and Fawcett, 1970; Fawcett et al., 1970; Dym, 1973; Vitale et al., 1973), production of an androgen-binding protein (ABP) (Hansson et al., 1973; French and Ritzen, 1973) and of a protein regulating FSH secretion (inhibin) (Steinberger and Steinberger, 1976). The idea of a steroid hormone-producing Sertoli cell is not new. Recent works show that this cell has enzymatic activity permitting the transformation of testosterone into dihydrotestosterone, androstenedione and androstanediols (Dorrington and Fritz, 1975; Tence and Drosdowsky, 1976; Welsh and Wiebe, 1976) and the conversion of testosterone into 17β-estradiol (Dorrington and Armstrong, 1975). Moreover, this cell may also transform progesterone into testosterone (Christensen and Mason, 1965;
Lacy et al., 1969; Hall et al., 1969; Tence and Drosdowsky, 1976). The physiological meaning of these syntheses has not been elucidated. The Sertoli cell ultrastructure of several adult mammals is known. Some authors have been more interested in typical formations such as intercellular junctions and Charcot-Böttcher's crystalloids. The Sertoli cell junctions joining adjacent Sertoli cells were principally described by Brökelmann (1963), Nicander (1967), Flickinger and Fawcett (1967), Dym and Fawcett (1970), Dierichs and Wrobel (1975), Bigliardi and Vegni Talluri (1976), Gilula et al. (1976), Russell (1977a) and Connell (1978); those joining Sertoli cells to germ cells were reported by Ross (1976, 1977), Ross and Dobler (1975), Wrobel and Dierichs (1975) and Russell (1977a, b, c). Charcot-Böttcher's crystalloids have been studied by Bawa (1963), Nagano (1966, 1968), Sohval et al. (1971) and Toyama (1975).

In this report, a comparative description of the Sertoli cell in immature, pubescent, adult and cryptorchid pigs is presented. The ultrastructure of this cell has been described in immature pig by Dierichs and Wrobel (1975) and Wrobel and Dierichs (1975) and in the boar by Osman and Plöen (1978); that of Charcot-Böttcher's crystalloids in the adult and cryptorchid pig by Toyama (1975).

**Material and methods.**

Pigs of the Large White breed (Sus crofa L.) were used. Twelve immature 6-week old animals, 4 pubescent 8-month old ones and 2 adults about 2 years old were studied. Moreover, abdominal testes were taken from unilateral cryptorchid pigs about 8 months old. These testes presented varying degrees of regression and weighed between

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**PLATE 1**

*Ultrastructure of the Sertoli cell in immature pig testis (6 weeks)*

(d: densification, DL: desmosome-like junction, ER: smooth endoplasmic reticulum, G: germ cell, IG: interchromatin granules, L: lipid droplet, Nu: nucleolus, S: Sertoli cell.)

**FIG. 1.** — General view. Note regular outline of nuclei, large masses of compact chromatin and supranuclear position of lipid droplets. × 10 000.

**FIG. 2.** — Prominent nucleolar complex: nucleolus is flanked by 2 lateral spheres of chromatin (asterisks). × 20 500.

**FIG. 3.** — Golgi apparatus grouping several dictyosomes. Note presence of coated vesicles (arrows). × 14 000.

**FIG. 4.** — Group of dark droplets. × 60 000.

**FIG. 5.** — Smooth ER in apical cell region. Note abundance of ribosomes × 20 800.

**FIG. 6.** — Junctions between 2 Sertoli cells. The dense material on plasma membrane is accompanied by cisternae of smooth ER oriented parallel to cell boundary. The region is rich in microfilaments. × 57 500.

**FIG. 7.** — Desmosome-like junctions between a Sertoli cell and a germ cell. × 63 000.
95 and 280 g (Dufaure et al., 1971). The samples were taken in the external region of the testis at castration or at slaughter. The testicular fragments were fixed by 2 p. 100 glutaraldehyde in 0.1 M Sörensen's phosphate buffer at pH 7.2 for 20 min., postfixed in 1 p. 100 osmic acid in the same buffer with 4.5 p. 100 sucrose added for 1 hr. 30 min., embedded in epon and cut with a LKB ultramicrotome. The sections were contrasted with alcoholic uranyl acetate and lead citrate. Observations were done with an 80 KV JEM 100 B electron microscope.

Observations.

6-week old animals. — Sertoli cells are all identical (Pl. I, fig. 1) and juxtaposed on the internal edge of the seminiferous cord; they are joined by lateral junctions. The apical regions are already forming numerous extensions.

The nucleus is always basal (Pl. I, fig. 1) and elongated. It has a regular shape; its internal membrane is accompanied by a discontinuous fringe of compact chromatin. The nucleoplasm is not dense and contains dispersed chromatin and some large masses of compact chromatin. Two spherical masses of diametrically opposed chromatin are associated with the nucleolus (Pl. I, fig. 2). A patch of interchromatin granules is often situated near the nucleolus (Pl. I, fig. 1). These granules are about 25 nm in diameter.

The narrow strip of cytoplasm under the nucleus contains only a few dark-matrix mitochondria with lamellar transversal cristae, some scattered small tubular elements of the smooth and rough endoplasmic reticulum (ER), free or polysome-grouped ribosomes, some small lipid droplets, and near the nucleus, some microfilaments 5 nm in diameter. Mitochondria of the same type are found in the lateral and apical regions as well as a Golgi apparatus formed by a group of dictyosomes surrounded by numerous coated vesicles (Pl. I, fig. 3, arrows), very abundant smooth ER (Pl. I, fig. 5) constituted of saccules organized in large concentric circles, many ribosomes (Pl. I, fig. 5), a large supranuclear lipid droplet (Pl. I, fig. 1), some small primary lysosomes and a group of small dark droplets 50 to 70 nm in diameter (Pl. I, fig. 4). The latter were always arranged in a circle around a homogeneous « matrix ».

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PLATE II

_Ultrastructure of pubescent and adult pig Sertoli cells._


FIG. 1. — Nucleus and perinuclear region of an A-cell. × 7 500.

FIG. 2. — General view of a B-cell. Note triangular nucleus, abundant smooth ER and junctions with a germ cell (arrows). × 8 400.

FIG. 3. — Piles of smooth ER in an A-cell. × 40 000.

FIG. 4. — Cytoplasmic region near the nucleus of an A-cell. Note numerous lysosomes. × 12 000.

FIG. 5. — Numerous microtubules near the cellular lateral membrane in an A-cell. × 27 500.
Sertoli cell junctions are seen facing an other Sertoli cell or a germ cell. Between adjacent Sertoli cells there are gap junctions and some formations (Pl. I, fig. 6) which develop where the intercellular space narrows from 25 to 10 nm. These formations are the result of a sub-surface modification. The thickness of the plaques formed at the junctional site is about 10 to 20 nm. In some cases these structures are accompanied, on the internal side, by short smooth ER cisternae (Pl. I, fig. 6). This region is particularly rich in microfilaments. Desmosome-like junctions are noted facing germ cells (Pl. I, fig. 7).

**Pubescent and adult animals.** — Two types of Sertoli cells (A) (Pl. II, fig. 1) and (B) (Pl. II, fig. 2), are found. A-cells are more numerous, extending from the basement to the tubule lumen. Their apical region is very irregular. The basal nucleus is elongated (Pl. II, fig. 1) and is oriented as in the immature pig. Deep, numerous indentations are observed and the nuclear content is light. Compact chromatin is only found in some small isolated masses. The spheric nucleolus shows no peculiarity; it has lost its two masses of associated chromatin. The interchromatin granules are grouped in one or two large patches (Pl. II, fig. 1). The nucleus is always surrounded by a cytoplasmic area particularly rich in microfilaments.

In the regions near the nucleus, round or ring-shaped mitochondria are seen having a matrix of variable density; the scarce cristae of these mitochondria are lamellar. The smooth ER is abundant and sometimes in the form of stacks of flat cisternae (Pl. II, fig. 3); rough ER is rare. Ribosomes and Golgi formations are less numerous than in the immature. Lysosomes vary in size and shape and have a heterogeneous content (Pl. II, fig. 4). Microtubules are either isolated or grouped, but always run parallel to the cellular membrane (Pl. II, fig. 5). The lipid droplet is not located above the nucleus but laterally (Pl. II, fig. 1). The dark droplets of 50 to 70 nm described

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**PLATE III**

*Sertoli A-cell ultrastructure in pubescent and adult pigs.*


**FIG. 1.** — Longitudinal section of Charcot-Böttcher’s crystalloids. Some are confluent; one is surrounded by smooth ER. They are located near the lateral cell membrane (arrows). × 30 000.

**FIG. 2.** — Smooth ER opposite the acrosome of an early spermatid. × 29 000.

**FIG. 3.** — Apical region of the cell. Note numerous microtubules and the composition of the Sertoli cell-elongated spermatid junction at spermatid head. × 38 000.

**FIG. 4.** — Cross-section of a specialized Sertoli cell junction. Microfilaments are scattered in groups throughout the plasma membrane (arrows) of every cell; these are accompanied by smooth ER. × 42 000.

**FIG. 5.** — Longitudinal section of specialized Sertoli cell junction. Microfilaments are lined up parallel to the plasma membrane (arrow). × 32 000.

**FIG. 6.** — Sertoli cell junctions in the basal region of the cell. Note smooth ER. × 32 000.

**FIG. 7.** — Desmosome-like junction linking a Sertoli cell to an early spermatocyte. × 30 000.
in the immature are also present. Charcot-Böttcher's crystalloids (Pl. III, fig. 1) form electron-dense groups longitudinally striated due to the parallel arrangement of short rectilinear microfilaments. These crystalloids of various sizes and orientations may be confluent. In some cases, they are surrounded by a flat saccule of smooth ER and are usually found near the lateral walls of the cell. The apical cytoplasm contains a large number of microtubules (Pl. III, fig. 3), lysosomes, some elongated or ring-shaped mitochondria and piles of smooth ER localized near the acrosomal region of each young spermatid (Pl. III, fig. 2).

The specialized Sertoli cell junctions (Pl. III, figs. 4, 5) consist of bundles of rectilinear microfilaments running parallel to the plasma membrane of each cell. Deep the layer of microfilaments are elongated cisternae of smooth ER (SSC : sub-surface cisternae) which often bear ribosomes on the side toward the cell body. The intercellular space in these structures decreases from about 25 to 10 nm. Other than these formations, there are junctions near the cell base resembling those described in immature animals (Pl. III, fig. 6). The Sertoli cells are joined to the sex cells by different systems, depending on the stage of gametogenesis. The early spermatocytes (preleptotene stage) are joined to Sertoli cells by desmosome-like junctions (Pl. III, fig. 7). Facing spermatocytes there are also junctions developing only within the Sertoli cell cytoplasm; these have a microfilamentary structure identical to that of the typical Sertoli cell junctions. These structures are found at different stages of spermatid formation. They are first limited to the lateral acrosome regions, and then form a continuous mantle covering the later spermatid head (Pl. III, fig. 3). This sheath is composed of microfilaments accompanied on the inner side by elongated cisternae of smooth ER which may contain scattered ribosomes on its surface.

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PLATE IV

Ultrastructure of cryptorchid pig Sertoli cells.


FIG. 1. — View of a light cell. Note regular-shaped nucleus, few cytoplasmic organelles. × 16 000.

FIG. 2. — An A-cell. Nucleus deeply indented, cytoplasm rich in varied organelles. × 7 000.

FIG. 3. — Specialized junction linking 2 Sertoli cells. Longitudinal section. × 34 000.

FIG. 4. — Superposition of different Sertoli cell junction systems; towards the base, junctions resemble those seen in the immature (solid arrows); above, typical specialized junctions (open arrows). × 10 000.

FIG. 5. — Cross-section of a voluminous Charcot-Böttcher's crystalloid accompanied by smooth ER. × 15 000.

FIG. 6. — Higher magnification of a part of the Charcot-Böttcher's crystalloid shown in the preceding figure. × 45 000.
Sertoli B-cells (Pl. II, fig. 2) are practically regular and are found only in the basement region of the seminiferous epithelium. The triangular-shaped nucleus is deeply indented; its content seems more electron-dense than that of the A-cell, although there is the same amount of compact chromatin. Cytoplasm volume is reduced and the cytoplasm contains numerous saccules of smooth ER, some mitochondria with lamellar cristae and a large quantity of unoriented microfilaments. The voluminous lipid droplet is present as in A-cells. Only desmosome-like junctions are observed developing at the early spermatocyte level (Pl. II, fig. 2, arrows).

**Cryptorchid animals.** — Sertoli cells have several aspects. Some present a light hyaloplasm (Pl. IV, fig. 1), while other more irregular ones resemble the A-cells noted in the normal mature testis (Pl. IV, fig. 2). Although typical B-cells are not found, some cells resemble them in form, surface occupied and nuclear aspect.

The light cells (Pl. IV, fig. 1) have a regular outline but the same structure as that of the adult A-cell. The cytoplasm contains smooth ER (sometimes vesicular), a small number of ribosomes and microfilaments and a reduced Golgi apparatus. Microtubules are only found near the lateral membranes of the cell. The same dark droplets observed in the other cases are also seen here. The mitochondria are few and have no peculiarity. The lipids form one or two voluminous droplets. The junctions with neighboring Sertoli cells are identical to those of the adult (Pl. IV, fig. 3). Between these elements and the cell base we find junctions identical to those described in the immature (Pl. IV, fig. 4).

The cells corresponding to A-cells have two peculiarities: abundant microtubules (Pl. IV, fig. 5) and numerous Charcot-Böttcher crystalloids; the latter may sometimes form a very voluminous aggregate (Pl. IV, figs. 5, 6), but are of the same structure.

**Discussion.**

The adult pig has a heterogeneous Sertoli cell population composed of two morphological cell types: A-cells, which are more numerous, and B-cells. A-cell nuclei, by their position, shape and chromatin, resemble those described in other species of mammals (Bawa, 1963; Brökelmann, 1963; Nagano, 1966; Dym, 1973, 1974; Fawcett, 1975). The configuration of the nucleolus is simpler than reported in rodents and ruminants (Fawcett, 1975) and in our observations under other physiological conditions (immature and hypophysectomized animals).

As in other species, the rough ER is limited to the basal part of the cell. In its smooth form, it occupies a considerable place and toward the interior of the cytoplasm constitutes large figures. The abundance of this organelle in pig, as in other mammals (Nagano, 1966; Dym, 1973; Fawcett, 1975), may be related to Sertoli cell steroid hormone production (Dorrington and Fritz, 1975; Dorrington and Armstrong, 1975; Tence and Drosdowsky, 1976; Welsh and Wiebe, 1976), the smooth ER being known to contain steroidogenic enzymes (Tamaoki, 1973). The smooth ER is also localized in the region adjacent to the developing acrosome in early spermatids; at that level it is less concentrated than noted in some species of artiodactyls and rodents (Fawcett, 1975). The smooth ER is always present in the specialized intercellular Sertoli-Sertoli and Sertoli-spermatid junctions, but its role is not known.

Lysosomes attest to the phagocytic action of Sertoli cells on degenerating germ cells and residual bodies (Sapsford et al., 1969; Black, 1971). A group of dark, regular-
shaped droplets was mentioned whose nature is unknown; their constant presence must have meaning and requires further study. Bawa (1963) described identical figures in man.

The lipids form a voluminous droplet characteristic of this species and of a wild African pig *Phacocheraeus aethiopicus* (Fawcett, 1975). Some authors interpret Sertoli lipids as a residue of Sertoli phagocytic action (Lacy et al., 1969). This hypothesis was also studied by Sapstord et al. (1969) with several slight differences. It should be noted that in the pig, this lipid droplet is found at the earliest stages before any spermatogenesis occurs. Lacy suggests that the droplets are used again to synthesize a substance acting on spermatogenesis (Lacy et al., 1969). Such lipid droplets are also regularly found in steroidogenic cells where they represent cholesterol pools (Sand et al., 1972).

The role of microtubules, particularly abundant in many species (Brökelmann, 1963; Bawa, 1963; Christensen, 1965; Flickinger, 1967; Dym, 1973) was for a long time limited to that of a rigid cellular cytoskeleton. According to Russell (1977b), microtubules participate in germ cell progression, then serve in remodelling the cell.

Charcot-Böttcher’s crystalloids were reported by Bawa (1963), Nagano (1966) and Sohval et al. (1971) in adult man and Toyama (1975) in the boar. According to Toyama, these structures result from microfilament overproduction in the basal cytoplasm. The crystalloids then migrate and aid in forming specialized junctions. Present observations agree with this hypothesis because the crystalloids are found near the plasma membrane, oriented in a way foreshadowing the formation of specialized Sertoli cell junctions.

The description of these junctions in pig (Toyama, 1975), rat (Gilula et al., 1976) and dog (Connell, 1978) is comparable to those of the present study. Toyama (1976) proved that microfilaments found in the junctions were composed of a substance similar to actin, thus indicating that they could play an active role in sex cell displacement (Toyama, 1976; Gilula et al., 1976; Nagano and Suzuki, 1976). Junctions between Sertoli cells and germ cells were reported by Ross and Dobler (1975) and by Russell (1977a, b, c). They observed on one hand desmosome-like junctions with early germ cells, and on the other characteristic junctions with spermatids; these latter were termed as « ectoplasmic specialization ». The same formations were observed in the boar.

B-cells are rare, but are easily distinguishable by their morphology and special situation. Although of reduced volume, their cytoplasm contains elements already noted in A-cells and often considered as characteristic of steroidogenic cells (smooth ER, lipid droplet). These cells are not degenerative.

Sertoli cell maturation may be estimated using two criteria: distribution of nuclear material and establishment of specialized junctions. At birth in the mouse, the nucleolus is associated to the nuclear membrane; at maturity, a nucleolar complex is formed containing 3 elements: a nucleolar body flanked by 2 chromatin satellites. In the pig we found this structure in immature, but not in pubescent animals. On the other hand, it is seen in the hypophysectomized pigs (Chevalier, in progress). Nucleolar evolution in relation to physiological state will be discussed later. The barrier formed by specialized Sertoli cell junctions does not exist at birth (Vitale et al., 1973; Nagano and Suzuki, 1976). The only junctions reported in the newborn are gap junctions; the number of these decreases progressively as that of typical Sertoli junctions increases.
This process occurs between days 16 and 19 in rat and mouse. In the pig, we could not determine the date this barrier was established. The immature 6-week old pig presents gap junctions and another junction type indicating that the barrier is in the process of forming.

The study of abdominal testes entirely or partially free of sex cells agrees with that of Gilula et al. (1976). These authors showed that Sertoli cell maturation does not result from interaction with sex cells since the testis barrier is found in young rat seminiferous tubules containing no germ cells. Considerable development of Charcot-Böttcher's crystalloids has been reported in cryptorchid pig (Toyama, 1975). This author supposes that these structures, which in the normal animal contribute to the development of specialized junctions, no longer serve a purpose and thus accumulate. This hypothesis contradicts the fact that specialized junctions in cryptorchid animals develop normally.

The presence of two morphological cell types has already been reported in man (Johnsen, 1969; Wartenberg, 1978), prepubescent pig (Wrobel and Dierichs, 1975) and rat in in vitro conditions (de Martino et al., 1977). The latter authors do not interpret this result. On the other hand, Wartenberg (1978) supposes a different embryonic origin and opposite roles in the control of meiosis (induction and repression). The A- and B-cells defined by Johnsen (1969) using histochemical methods seem to correspond, respectively, to the cells to which we have given the same names. That author shows their existence in young human, adult human and in subjects suffering from different spermatogenetic disorders. Two cell types were not found in the young 6-week old pig. In the cryptorchid animal, on the contrary, cell polymorphism was evident. These variations, apparently related to different physiological conditions, should be studied in the light of Wartenberg's hypothesis.

In conclusion, the type A Sertoli cell in normal adult pig has the main ultrastructural characteristics found in other mammalian species. It will be fascinating to discover the meaning of the existence of the two morphological cell types. Different experimental models will be used to study this problem. We will present the ultrastructure of the hypophysectomized pubescent pig Sertoli cell in a future report.

Reçu en février 1978.
Accepté en mai 1978.

Résumé. Chez le jeune, toutes les cellules de Sertoli sont morphologiquement identiques. Elles contiennent un RE lisse abondant et de nombreux ribosomes. Les premières jonctions spécialisées (Sertoli-Sertoli) sont en cours de formation à 6 semaines.

Il existe chez le pubère et l'adulte deux formes de cellules de Sertoli. Les cellules de Sertoli typiques (cellules A) ont un noyau clair allongé. Elles s'étendent de la base du tube à la lumière et leur structure est identique à celle décrite chez d'autres espèces (nombreux microfilaments et ribosomes, RE lisse abondant, microtubules orientés parallèlement, cristalloïdes de Charcot-Böttcher, jonctions spécialisées). Les autres cellules (cellules B) sont moins hautes avec un noyau sombre de forme triangulaire et un cytoplasme de volume réduit contenant du RE lisse et de nombreux microfilaments. Les deux types de cellules sont caractérisés par la présence d'un volumineux globule lipidique.

Plusieurs formes de cellules sont décelables chez le cryptorchide. Des cellules claires dont la plupart des organites se sont raréfiés. Des éléments assimilables aux cellules A de l'adulte mais avec une grande quantité de microtubules et des cristalloïdes de Charcot-Böttcher qui peuvent former des structures très importantes. Les cellules de type B n'existent pas mais quelques éléments les rappellent par leur volume et l'aspect de leur noyau.
References


