Reproductive Biology and Endocrinology

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Review

Understanding spermatogenesis is a prerequisite for treatment

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Abstract

Throughout spermatogenesis multiplication, maturation and differentiation of germ cells results in the formation of the male gamete. The understanding of spermatogenesis needs detailed informations about the organization of the germinal epithelium, the structure and function of different types of germ cells, endocrine and paracrine cells and mechanisms, intratesticular and extratesticular regulation of spermatogenesis. Normal germ cells must be discriminated from malformed, apoptotic and degenerating germ cells and tumor cells.

Identification of the border line between normal and disturbed spermatogenesis substantiate the diagnosis of impaired male fertility. The profound knowledge of the complicate process of spermatogenesis and all cells or cell systems involved with is the prerequisite to develop concepts for therapy of male infertility or to handle germ cells in the management of assisted reproduction.

Introduction

Starting from a self-renewing stem cell pool, male germ cells develop in the seminiferous tubules of the testes throughout life from puberty to old age. The complete process of germ cell development is called spermatogenesis. Subdivisions are spermatogoniogenesis, meiosis, spermiogenesis, spermiation.

The products of spermatogenesis are the mature male gametes, namely the spermatozoa. The light microscopical evaluation of the ejaculate permits evaluation of the number of spermatozoa, shape and motility patterns and assessment of other cellular components. All these provide the first information about the success of spermatogenesis [1].

A reduced number of spermatozoa, predominating malformed spermatozoa or reduced and inefficient motility may be the cause for disturbed fertility or infertility of a patient.

Yet, the standard evaluation of the ejaculate does not provide in many cases sufficient information about the defects of spermatogenesis. A more thorough evaluation of the ejaculate may disclose a variety of disturbances originating in the different steps of spermatogenesis and may shed light on disturbed testicular functions or even disclose in the presence of early testis cancer.

Biopsies of the testes may be necessary to obtain valid informations about the quality of spermatogenesis or for exclusion of early testis cancer.

Spermatogenesis depends from intratesticular and extratesticular hormonal regulatory processes and functions of the intertubular microvasculature, the Leydig cells and other cellular components of the intertubular space.

The complex situation may be elucidated step by step:

Organisation of the testis

The human testes are two organs of the shape of rotation ellipsoids with diameters of 2.5 × 4 cm engulfed by a capsule (tunica albuginea) of strong connective tissue [2]. Thin septula testis (Fig. 1A) divide the parenchyma of the testis in about 370 conical lobules. The lobules consist of the seminiferous tubules and intertubular tissue, containing groups of endocrine Leydig cells and additional cellular elements. The seminiferous tubules are coiled loops (Fig. 1B). Their both ends open into the spaces of the rete testis [3]. The fluid secreted by the seminiferous tubules is collected in the rete testis and delivered to the excurrent ductal system of the epididymis.

Structure of the seminiferous tubule

The seminiferous tubule consists of the germinal epithelium and the peritubular tissue (lamina propria)(Fig. 1C) [4]. The mean diameter of a seminiferous tubule is about 180 μm, the height of the germinal epithelium measures 80 μm and the thickness of the peritubular tissue is about 8 μm. The germinal epithelium (Fig. 2A) consists of cells that include different developmental stages of germ cells, namely spermatogonia, primary and secondary spermatocytes and spermatids. These are located within invaginations of Sertoli cells.

The prismatic Sertoli cells are connected by specialized zones of tight junctions of cellular membranes separating the germinal epithelium in a basal and an adluminal compartment (Fig. 2B). These specialised zones, the so-called "tight junctions" form the blood-testis barrier of the testis. During maturation the germ cells pass this barrier entering the adluminal compartment where they find protection from diffusion of extraneous substances.

Sertoli cells, investigated in histological sections, exhibit increasing amounts of lipid droplets in correlation to advanced age being an indicator of the "biological clock" of the testis (Fig. 3A)[1].

Further functions are attributed to Sertoli cells [5]: 1. Sustentacular and nutritive functions for the germ cells. 2. Organization of the delivery of mature spermatids into the tubular lumen (spermiation). 3. Production of endocrine and paracrine substances for the regulation of spermatogenesis. 4. Secretion of androgen binding protein (ABP) for the maintenance of epithelia of the excurrent duct system. 5. Interaction with the intertubular endocrine Leydig cells.

The peritubular tissue (lamina propria of seminiferous tubules) consists of about five layers of myofibroblasts with intermingled connective tissue ground substance. The myofibroblasts cause peristaltic contractions of the seminiferous tubule giving rise to transport of the immotile spermatozoa to the rete testis [6]. The thickness of the peritubular tissue normally is about 8 μm. In cases of disturbed spermatogenesis the peritubular tissue may be thickened by connective tissue ground substance up to 12 μm and more.

Spermatogenesis

Spermatogenesis is the process by which a complex, interdependent population of germ cells produces spermatozoa [7]. Spermatogenesis begins at puberty after a long preparatory period of "prespermatogenesis" in the fetus and the infant. Three major stages can be distinguished: spermatogoniogenesis, maturation of spermatocytes and spermiogenesis, which is the cytodifferentiation of spermatids.

Spermatogoniogenesis

Several types of spermatogonia are distinguished by their position in the basal part of the germinal epithelium, their morphology and stainability of nuclei: A pale type-, A dark type- and B type-spermatogonia [8]. A type spermatogonia belong to the stem cell pool of spermatogenesis. B type-spermatogonia represent the onset of germ cell development up to spermatids.

Spermatogonia multiplicate continuously in successive mitoses. Spermatogonial cell divisions are usually incomplete. The daughter cells remain interconnected by cytoplasmic bridges so that a clone derived from one stem cell forms a syncytium of cells. Syncytial connections are maintained through spermatogonial and spermatocytic stages and are dissolved only in advanced phases of spermatid development. It is thought that the formation of these clones are the basis for the synchronuous development of germ cells (Fig. 3B).

Both A type spermatogonia are necessary for intact spermatogenesis. In reduced spermatogenesis A dark-type spermatogonia are often absent. Of course, in the absence of both types of spermatogonia, no spermatogenesis takes place and the germinal epithelium consists of Sertoli cells only. Spermatogonia may be absent from birth (congenital Sertoli cell-only Syndrome) or may be destroyed by different noxes, e.g. x-radiation, (acquired Sertoli cell-only Syndrome). In cases of disturbed ability of spermatogonia to develop B-type spermatogonia the number of A pale type spermatogonia increases and bi- or multilayered groups (Fig. 4A) of spermatogonia in the basal compartment are formed without further developed germ cell stages. This aspect represents an arrest of spermatogenesis at the stage of spermatogonia (Fig. 4B)[9]. The barrier of Sertoli cells can not normally be passed by A type-spermatogonia. Under special conditions, e.g. intratubular tumor cells, the barrier is interrupted and spermatogonia are

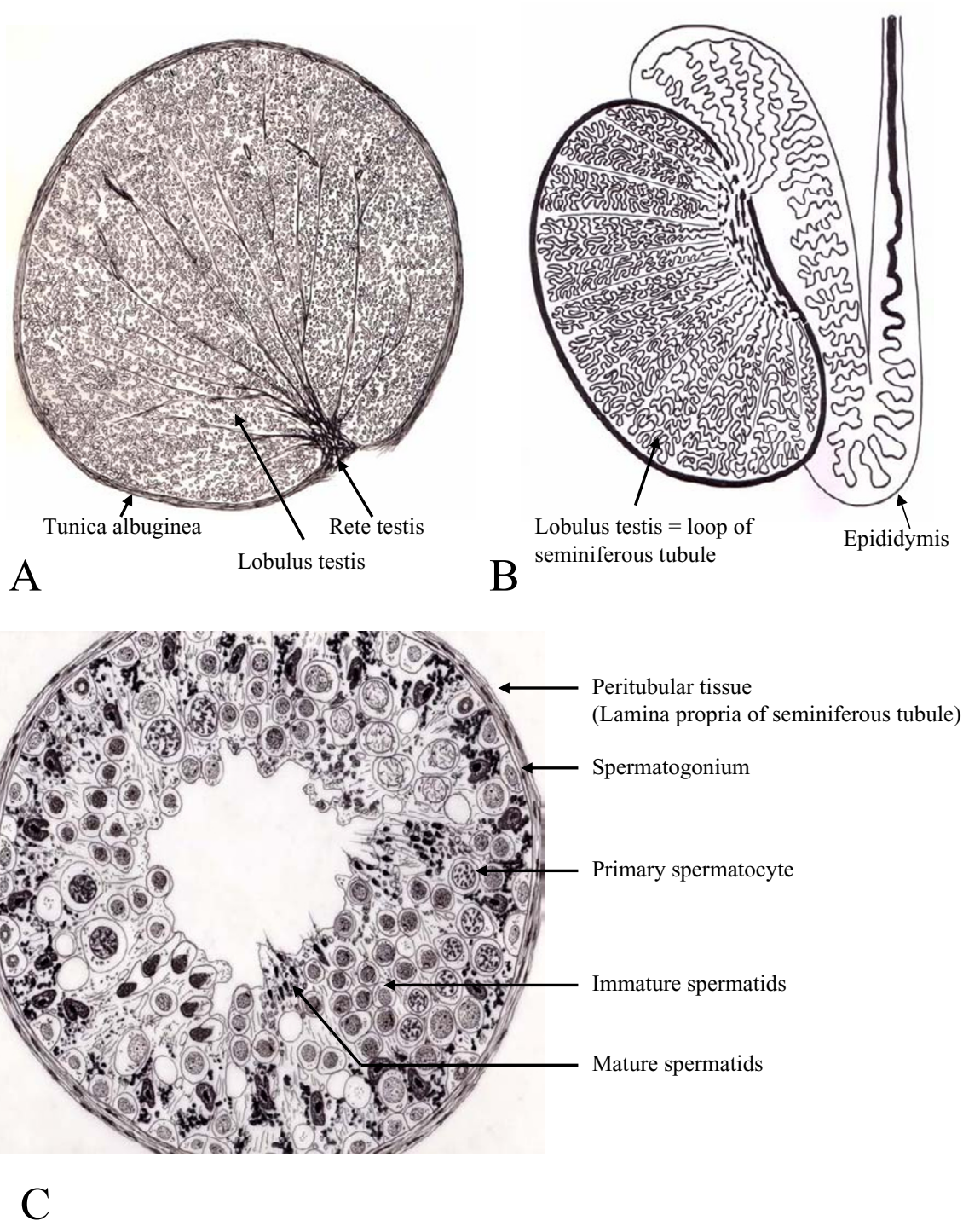


Figure 1
(A) Cross-section of the human testis. Drawing of a paraffin section. × 2.5. (B) Arrangement of the seminiferous tubules in the human testis and of the excurrent ductular system of the epididymis. Semi-schematic drawing. (C) Cross section of a seminiferous tubule of a fertile man 32 years of age. Drawing of a semithin section. × 300.

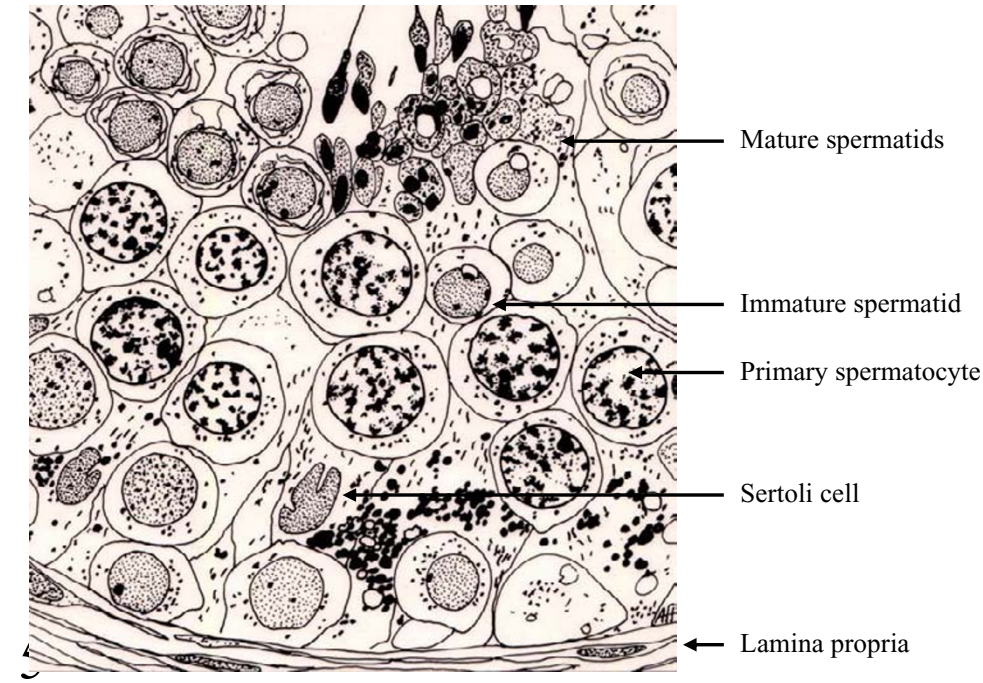
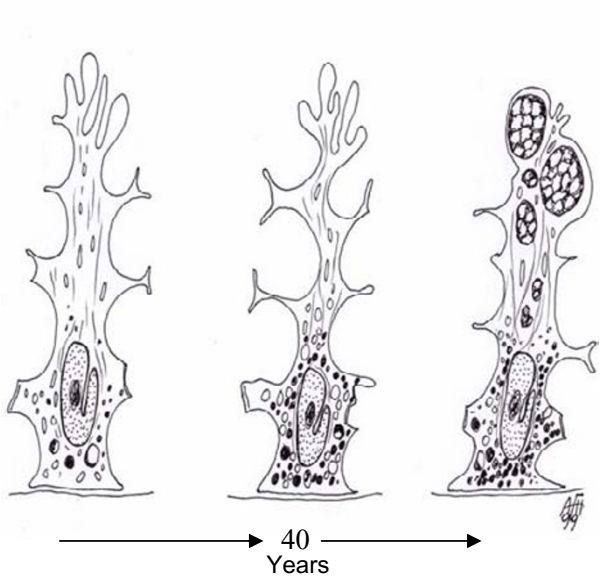


Figure 2
(A) Sector of the germinal epithelium in the seminiferous tubule. Drawing on the basis of a semithin section. × 900 (B) Sertoli cells divide the germinal epithelium in a basal and adluminal compartment. Arrows indicate the transport of substances only to the basal compartment, via the Sertoli cell into the adluminal compartment, via the Sertoli cell into the lumen.



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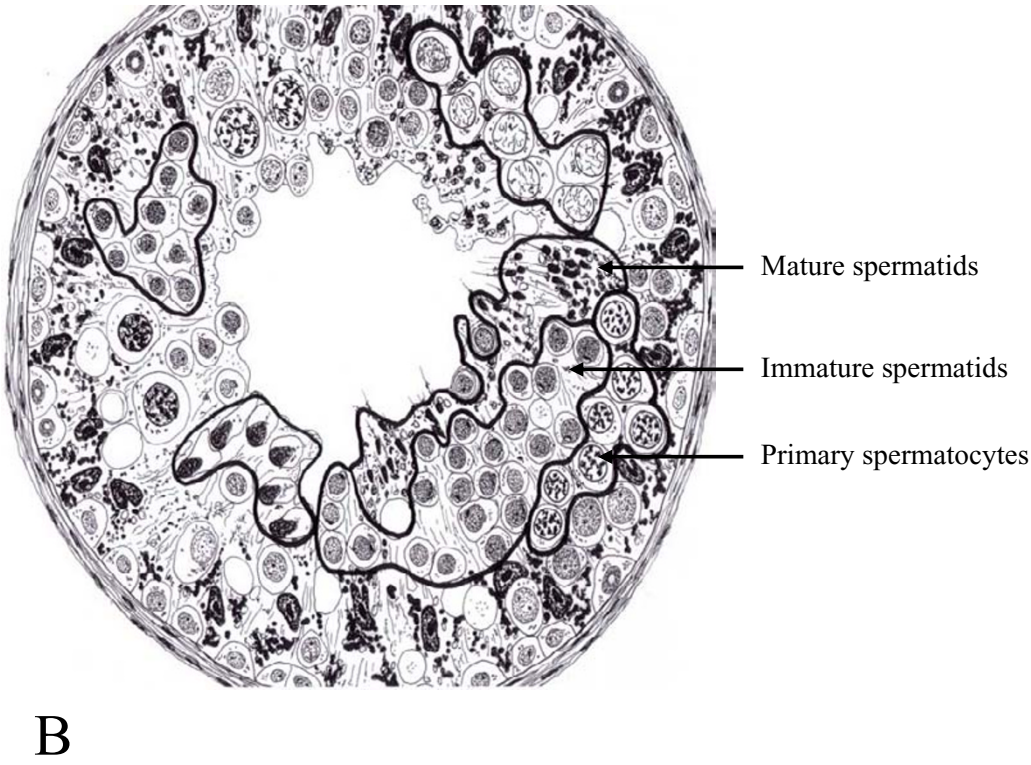

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Figure 3
(A) The storage of lipid droplets of different size and composition in the Sertoli cells correlates to the age of the man. The Sertoli cell represent a "biological clock" of the testis. (B) Seminiferous tubule with marked clones of germ cells. Drawing on the basis of a semithin section. × 300

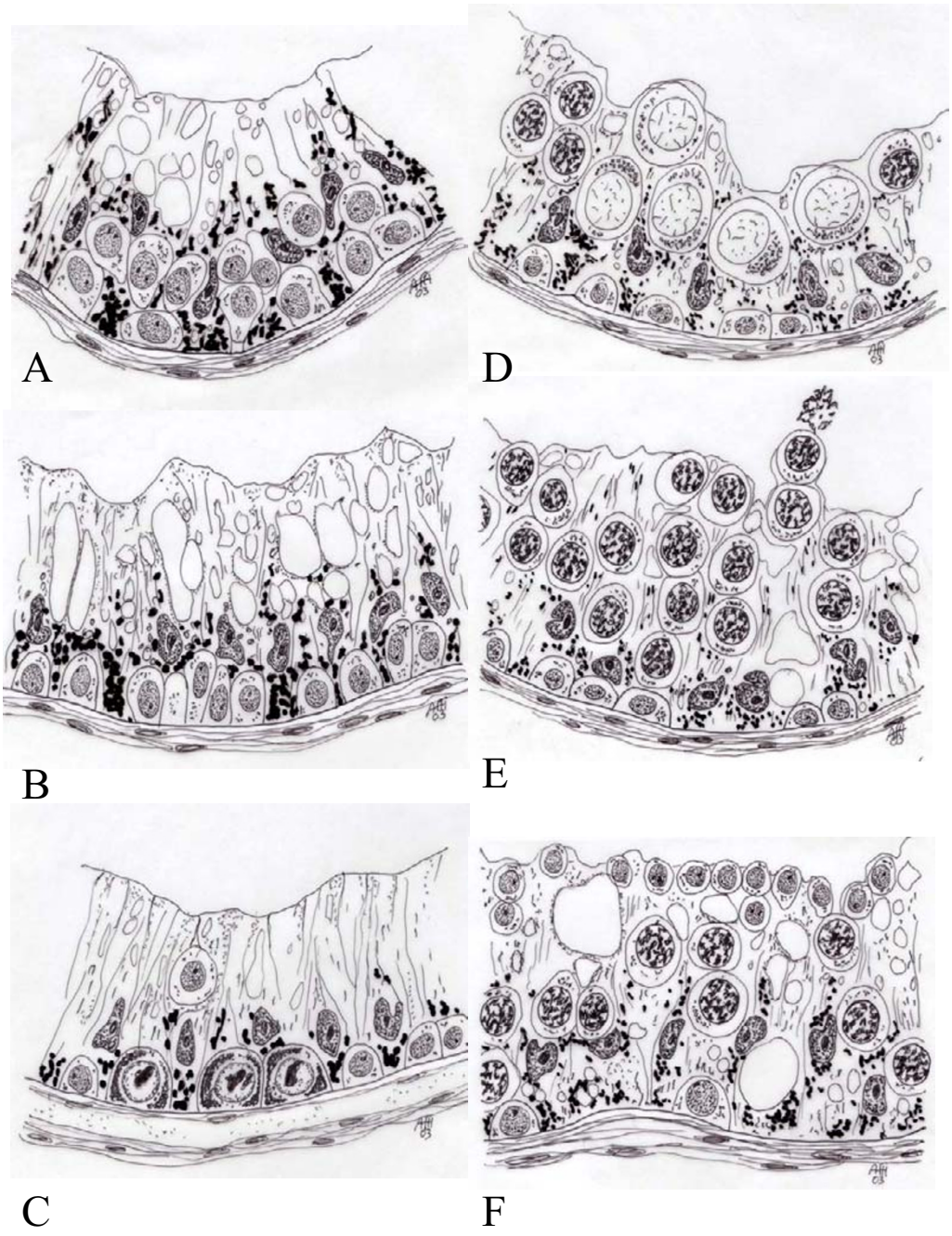


Figure 4
(A) Section of the germinal epithelium with multilayered spermatogonia. 52 years old infertile patient with arrest of spermatogenesis at the stage of spermatogonia. Drawing on the basis of a semithin section. × 600 (B) Arrest of spermatogenesis at the stage of A pale type-spermatogonia. 37 years old infertile patient with arrest of spermatogenesis at the stage of spermatogonia. Drawing on the basis of a semithin section. × 600 (C) Tumour cells in the basal compartment of the germinal epithelium dislocate a pale type-spermatogonia. 33 years old patient. Drawing on the basis of a semithin section. × 600 (D) Megalospermatocytes do not complete meiosis. 37 years old patient with impaired fertility. Drawing on the basis of a semithin section. × 600 (E) Arrest of spermatogenesis at the stage of primary spermatocytes. 34 years old patient with impaired fertility. Drawing on the basis of a semithin section. × 600 (F) Arrest of spermatogenesis at the stage of immature spermatids. 52 years old patient with impaired fertility. Drawing on the basis of a semithin section. × 600.

dislocated into the adluminal compartment where they disintegrate [10].

In the basal compartment of the seminiferous tubules tumour cells may be found. In semithin sections they differ noticeable from spermatogonia because of the their larger size, the prominent nucleolus, increase glycogen content and a clear peripheral border (Fig. 4C)[11]. In paraffin sections the detection of single tumour cells may be difficult and the PLAP (Placental alkaline phosphatase)-reaction is required to demonstrate a characteristic dark border [12]. Occasionally hypospermatogenesis is caused by the presence of neoplastic cells in the basal compartment of the germinal epithelium along the basal lamina. These basally situated neoplastic cells in the seminiferous tubules are characteristic of carcinoma-in-situ. They appear to be the stem cell population for most germ cell tumours including both seminomatous and teratomatous tumour types. Sporadic tumour cells may be found within tubules in association with active spermatogenesis, but as the neoplastic cells increase in number, spermatogenesis ceases and the remaining spermatogonia become detached and are released into the tubular lumen. After further proliferation of the neoplastic cells, these also appear in the lumen of the tubule or penetrate the peritubular tissue giving rise to the development of intertubular tumour cell clusters.

Meiosis of spermatocytes

The stage of meiosis is manifested through changes in chromatin configuration in the nucleus after the last spermatogonial division. Cells in meiosis are called spermatocytes. As the process of meiosis comprises two divisions, cells before the first division are called primary spermatocytes and before the second division secondary spermatocytes.

The primary spermatocytes are the largest germ cells of the germinal epithelium (Fig. 1C). The aspect of their nuclear chromatin represents the meiotic stages. Meiosis of spermatocytes starts with the leptotene stage of prophase already in the basal compartment of the germinal epithelium. After passing the Sertoli cell barrier, spermatocytes reach the adluminal compartment and continue with the further prophase stages, namely the zygotene stage, the pachytene and the diplotene stage. During the prophase the reduplication of DNA, the condensation of chromosomes, the pairing of homologuous chromosomes and the "crossing over" take place. After division the germ cells become secondary spermatocytes. They undergo no DNA-replication and divide quickly to the spermatids. The two maturation divisions of each spermatocyte result in four haploid cells, namely the spermatids. These differentiate into mature spermatids, a process called spermiogenesis which ends when the cells are released from the germinal epithelium. At this point, the free cells are called spermatozoa.

Many defects of meiosis are known indicating the vulnerability of this complicate process. Apoptotic spermatocytes are frequent. In some cases very large spermatocytes, so called megalospermatocytes (Fig. 4D) [13] appear. In these cells asynapsis of homologuous chromosomes occurs and the cells become abortive. Genetic disorders may cause these defects. Often, arrest of spermatogenesis at the stage of primary spermatocytes appears without any special aspect of changed morphology of the cells. The primary spermatocytes border the lumen of the seminiferous tubule and do not develop further (Fig. 4E). They disintegrate and spermatids are missing.

Spermiogenesis

During the cytodifferentiation of spermatids the following three processes take place: (fig 5A)

• Condensation of the nuclear chromatin to about one tenth of the volume of an immature spermatid

• Formation of the enzyme filled acrosome cap by the Golgi apparatus and its attachment to the nucleus

• Development of flagellum structures and their implantation to the nucleus.

The spermatids develop thus the configuration which enables them to leave the germinal epithelium during a complex process, called spermiation.

In summary, the differentiation of spermatids may be divided into 8 steps, demonstrated in figure 5A[14].

Normally, a large number of spermatids is malformed. Malformations may affect only the acrosome, the nucleus or flagellum or may be combined thus sometimes producing bizarrely abnormal spermatozoa. They are abortive germ cells.

A large variety of malformed spermatids may develop:

Malformations of the acrosome, absence of acrosome in cases of round-headed spermatids, disturbances of nuclear condensation, malformations of the flagellum, absence of parts of the flagellum, e.g. the middle piece, appearance of multinucleated spermatid giant cells and more (Figs. 5B, 5C, 6A, 6B, 6C, 6D) [9].

Spermiation

The delivery of mature spermatids from the germinal epithelium (spermiation) is managed by the Sertoli cells. As a result of the complex cooperation of intermediate

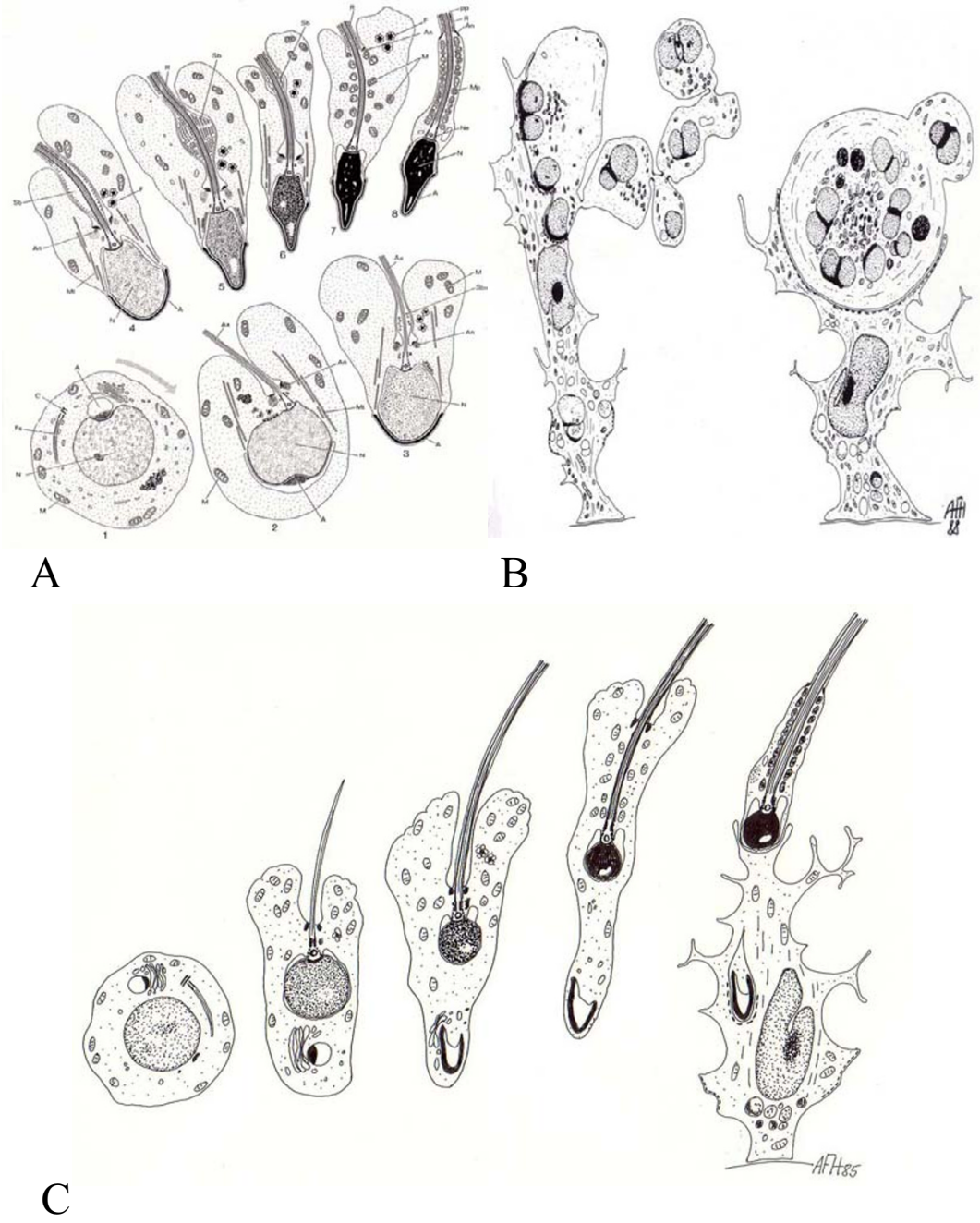


Figure 5
(A) Steps of spermatid differentiation: (1) Immature spermatid with round shaped nucleus. The acrosome vesicle is attached to the nucleus, the tail anlage fails contact to the nucleus. (2) The acrosome vesicle is increazed and flattened over the nucleus. The tail contacted the nucleus. (3–8) Acrosome formation, nuclear condensation and development of tail structures take place. The mature spermatid (8) is delivered from the germinal epithelium. Semi-schematic drawing on the basis of electron micrographs. From Ref. [14]. (B) Development of a giant spermatid by confluence of double headed spermatids of a clone. The giant spermatid remains in contact with the Sertoli cell. Drawing on the basis of electron micrographs. From Ref. [9]. (C) Differentiation of acrosomeless spermatozoa. The nuclear condensation and the development of tail structures is not disturbed. The acrosome, however, fails to establish contact to the nucleus of the spermatid and remains in the Sertoli cell cytoplasm. Drawing on the basis of electron micrographs. From Ref. [9].

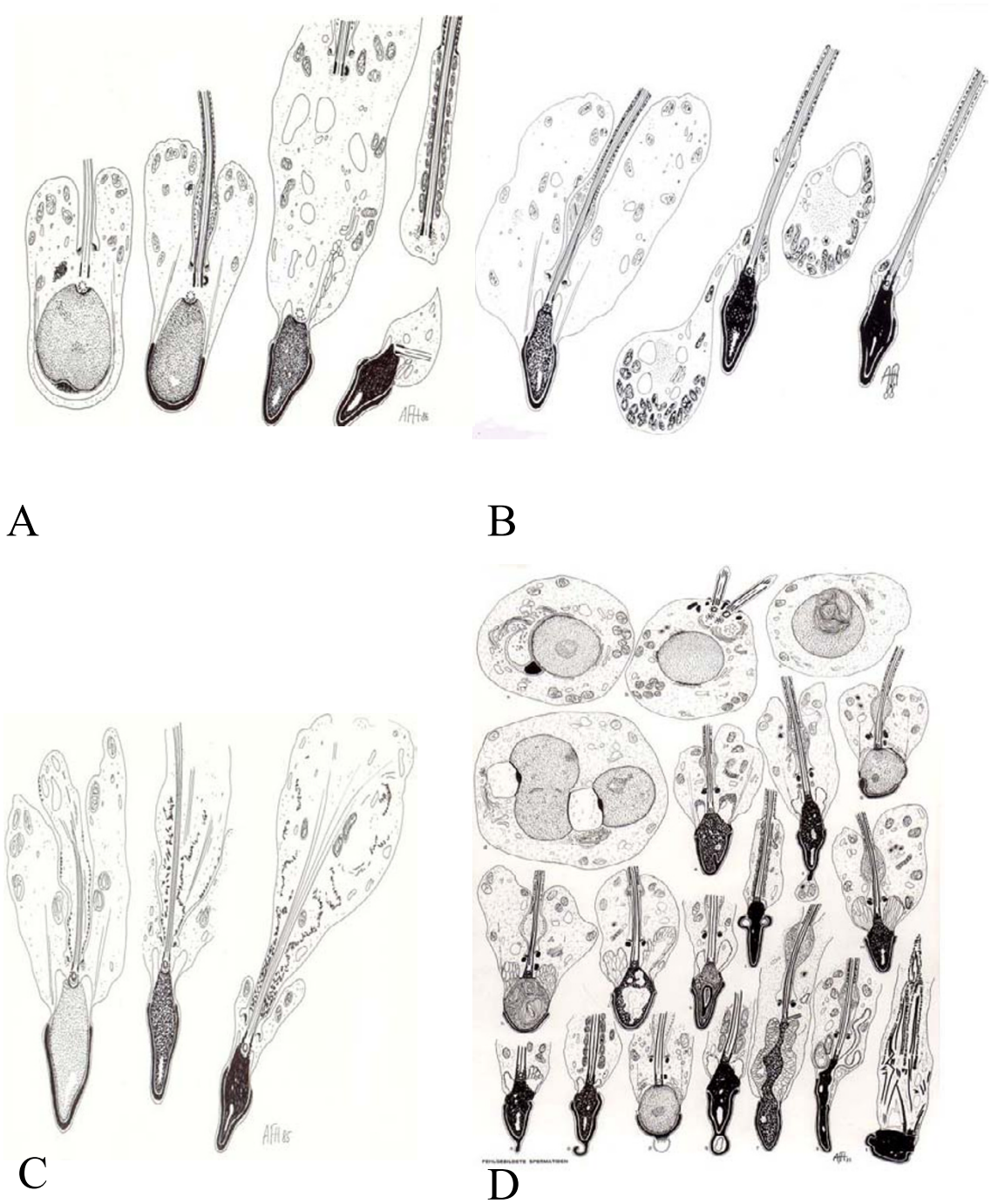


Figure 6
(A) Development of headless spermatozoa. Only the proximal centriole contacts the basal plate of the nucleus of the spermatid. The distal centriole is separated and develops the headless flagellum. Drawing on the basis of electron micrographs. From Ref. [9]. (B) Loss of the mitochondrial sheath during spermiogenesis. The spermatozoon is immotile. Drawing on the basis of electron micrographs. From Ref. [9]. (C) Development of malformed fagellar structures. Drawing on the basis of electron micrographs. From Ref. [9]. (D) Multiple malformations of spermatids. Drawings on the basis of electron micrographs from testicular specimen of several patients aged 43–85 years. The testes were removed by surgery as an additive treatment of prostatic cancer.

filaments and cytoplasmic tubules of the Sertoli cells spermatids are advanced to the border of the lumen of the seminiferous tubule [5]. There the mature spermatids close their intercellular bridges, disconnect their contact to the germinal epithelium and become free cells, now called spermatozoa. Smaller parts of the spermatids with cumulated RNA granules, a few mitochondria, lipid droplets and membranes are released, forming the so-called residual bodies. Most of them are incorporated and digested by the Sertoli cells [15].

Characteristics of normal spermatogenesis on the basis of histological sections

- Diameter of the seminiferous tubule 180 μm at the minimum

- Presence of A pale type-, A dark type-, B type-spermatogonia

- Presence of primary and secondary spermatocytes

- Differentiation of spermatids

- Zones of spermiation

- Score count of 8 at the minimum (see section - "Score count for the evaluation of spermatogenesis").

- Lumen of the seminiferous tubule

- Normal lipid distribution in the Sertoli cell cytoplasm

- Presence of stages of spermatogenesis

- Formation of clones of germ cells

- Thickness of the lamina propria of the seminiferous tubule of 8 μm at the maximum

-Normal structure and distribution of Leydig cells

Components of the intertubular space

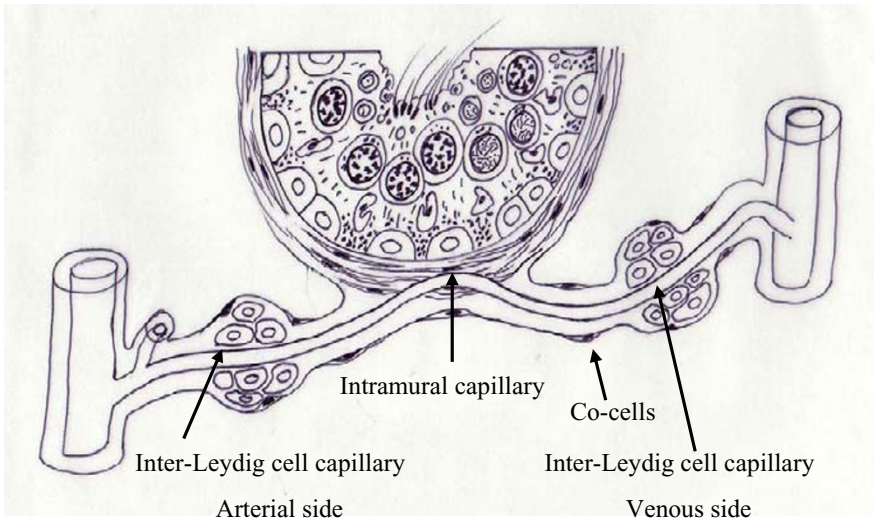
The intertubular space of the human testis contains the microvasculature, the endocrine Leydig cells, nerve fibres, macrophages, fibroblasts, further connective tissue cells (Co-cells) compartmentalizing in part this space and lymph vessels.

*Microvasculature*of the intertubular space is divided into inter-Leydig cell-capillaries of the arterial side, intramural capillaries in the peritubular wall of the seminiferous tubule and inter-Leydig cell capillaries of the venous side (Fig. 7A) [16]. The microvasculature accesses the seminiferous tubules and Leydig cells and permits distribution of endocrine and paracrine substances. Leydig cells ensheath parts of the microvasculature and deliver hormones into the vessels.

Under pathological conditions the intima of capillaries may be thickened thereby narrowing the lumen. Another aspect is the apposition of outer layers of the capillary wall by additional connective tissue ground substance [9]. In both cases the blood flow may be reduced. In consequence of this aspect of patchy arteriosclerosis focal degeneration of testicular tissue appears. This finding is frequently observed in men with oligozoospermia of uncertain genesis.

*Leydig cells*are prominent cells of the intertubular space [17]. They constitute groups surrounding the capillaries. Leydig cells produce and secrete among others androgens, the male sex hormone, the most well known of which is testosterone. Testosterone activates the hypophyseal-testicular axis, the masculinization of the brain and sexual behaviuor, the initiation, processing and maintenance of spermatogenesis, the differentiation of the male genital organs and secondary sex characteristics.

Recent investigations elucidated that the Leydig cells possess neuroendocrine properties in addition to their endocrine functions. There is evidence that Leydig cells express serotonin, catecholamine synthesizing enzymes, different antigens characteristic for nerve cells as well as neurohormones and their receptors, neuropeptides, cell adhesion molecules, components of the NO/cGMP-system, components of the renin/angiotensin system, neurofilament proteins, synaptic and storage vesicle proteins, and numerous growth factors and their receptors. Furthermore, Leydig cells possess antigens characteristic for glial cells such as astrocytes, oliogodendrocytes and Schwann cells. All these features characterize the Leydig cells as non-dividing, post-mitotic neuroendocrine cells with pluripotent properties. Some of the neural antigens (e.g. substance P, NO, C-type natriuretic peptide, catecholamines, IGF-1, TGF-β, PDGF) are involved in autocrine and/or paracrine regulation mechanisms of the testes [18] such as testosterone production and release, maintaining of the hypothalamo-hypophyseal-gonadal axis, the local communication between the somatic cells of the organ (Leydig cells, peritubular cells, Sertoli cells), the regulation of blood flow in the testicular blood vessels and the permeability of hormones and nutritive substances, as well as the contractility of seminiferous tubules and of the testicular capsule (tunica albuginea). Other substances discovered are seemingly rudiments that have been active during testis morphogenesis. The Leydig cells now are considered a part of the general neuroendocrine cell system [18,19].



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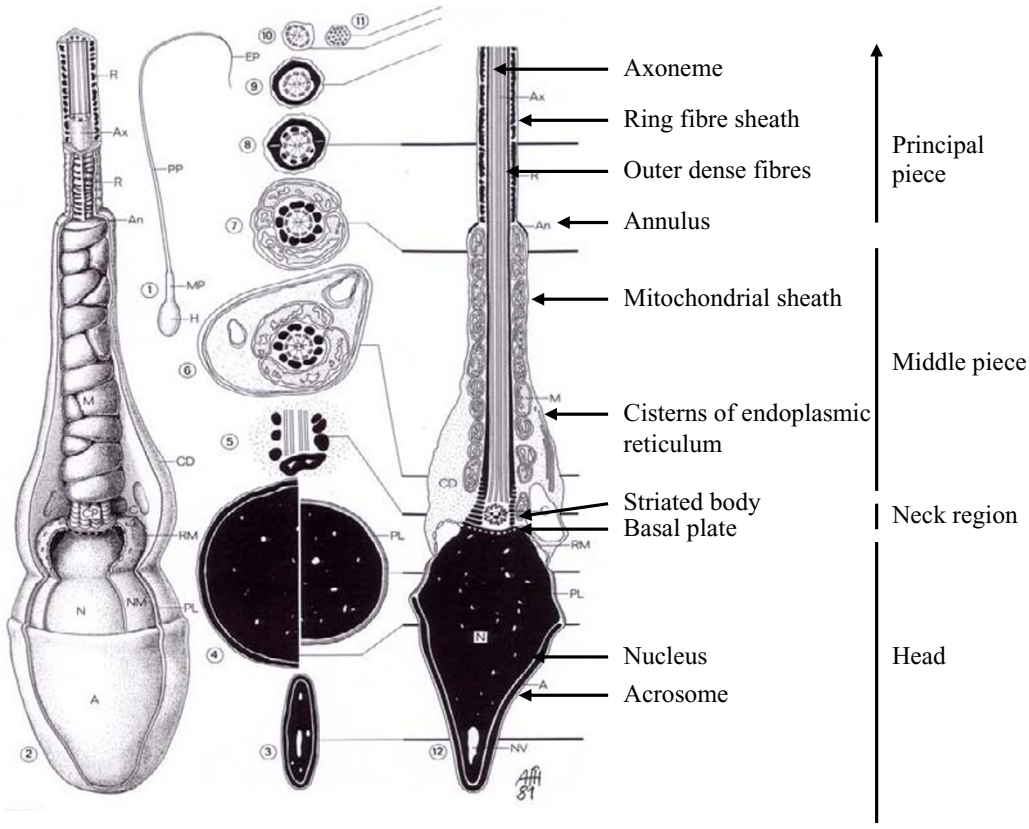

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Figure 7
(A) Microvasculature of the intertubular space. Co-cells encase the capillaries, Leydig cells and the seminiferous tubule. Modified from Ref. [20]. (B) The human spermatozoon. (1) Light microscopical aspect, (2) Virtual preparation of the spermatozoon showing the acrosome, the nucleus and nuclear envelopes, the mitochondrial sheath of the main piece of the flagellum, (3–11) Cross sections of the human spermatozoon of different levels indicated in (12) longitudinal section of the human spermatozoon. Semi-schematic drawing on the basis of electron micrographs. From Ref. [14].

Many different aspects of Leydig cell organization and function like degeneration, hyperplasia, or tumorous degeneration appear commonly along with disturbances of spermatogenesis. The number of Leydig cells does not necessarily correlate with hormone production. It has been shown by immunohistochemical investigations that in cases of an increased number of Leydig cells testosterone production takes place in few Leydig cells only.

*Fibroblasts*are randomly distributed in the intertubular space. In part they engulf groups of Leydig cells, capillaries and seminiferous tubules and represent compartmentalizing cells (Co-cells) (Fig. 7A). Immunocytochemical investigations indicate that Co-cells express antigens characteristic not only for fibroblasts but also for glia cells [20].

Larger bundles of *nerve fibres*commonly follow the septula testis for innervation of blood vessels, but are also encountered in the intertubular space. They cross groups of Leydig cells and in part contact seminiferous tubules. The function of these fibres is still a matter of debate.

*Macrophages*are a normal constituent of the intertubular space. Single cells are attached to the seminiferous tubules, Leydig cells or partly to blood vessels. Under conditions of inflammation or testicular cancer, the number of macrophages is increased. Morphologically different immigrant macrophages appear. Macrophages are able to enter the lumen of seminiferous tubules and to phagocytose spermatozoa [21,22].

*Free lymphocytes*are normally missing from the intertubular space of the human testis. Under special conditions, e.g. degeneration of seminiferous tubules, infections, allergic reactions and tumor cumulated free lymphocytes (infiltrates) are present.

*Lymph vessels*in the human testis are only found in the septula testis [23] and very seldom in the intertubular space. This organization differs completely from the condition in laboratory animals, e.g. rats, where lymphatics are a main component of the intertubular space [24].

Kinetics of spermatogenesis

Spermatogenesis commences during puberty and continues throughout life and until old age because of the inexhaustible stem cell reservoir. An abundance of germ cells are developed and delivered from the seminiferous tubules. The process of spermatogenesis is highly organized: Spermatogonia divide continuously, in part remaining spermatogonia, in part giving rise to spermatogenesis. Originating from dividing spermatogonia, cell groups migrate from the basal to the adluminal position of the germinal epithelium. Cell groups of different development are met in a section of a seminiferous tubule and contribute to the typical aspect of the germinal epithelium. Six of these typical aspects were described in the human testis as "stages of spermatogenesis". In any given region of the germinal epithelium every 16 days the same typical aspects of germ cell groups appear. This space of time is called "cycle of the seminiferous epithelium" [25]. The development of an A type spermatogonium up to mature spermatids requires 4,6 cycles, e.g. 74 days. The mature spermatids delivered from the germinal epithelium as spermatozoa are transported through the epididymal duct system during additional 12 days. Therefore, 86 days at the minimum must be calculated for a complete spermatogenetic cycle from spermatogonium to mature spermatozoa.

The products of spermatogenesis: spermatozoa

Spermatozoa with their unique shape are suitable for the transport to the female gamete. For this reason the nucleus of the spermatozoon is condensed, covered by an acrosome for establishing contact to the female gamete and connected with a flagellum for progressive motility. The diameter of the head of spermatozoon is 4–5 μm, the diameter of the flagellum is of 1–2 μm and the length of the spermatozoon measures 60 μm. The morphology of the human spermatozoon is depicted in figure 7B[14].

Spermatozoa acquire their competence of motility during the transport troughout the epididymal ducts. Different processes of the maturation of membranes and surface coat substances take place.

Principally, the motility of spermatozoa depends from normal development of the axoneme structures (e.g. microtubule doublets, dynein arms, etc.), the presence of mitochondrial sheath and the implantation of the flagellum at the nucleus by the both centrioles. Missing parts of the flagellar structures (e.g. dynein arms of the axoneme) give rise to immotility.

Efficiency of spermatogenesis

Spermatogenesis is a process of redundancy and of little efficiency in terms of quality management: Germ cell loss during spermatogenesis and the number of malformed spermatozoa in the ejaculate are extremely high. Calculating the potential spermatogenetic capacity of a testis with 100%, it must be realized that 75% of the developed germ cells are lost by apoptosis or degeneration. Only 25% of the germ cells reach the ejaculate and more than half of them are malformed. Therefore, only 12% of the spermatogenetic potential is available for reproduction.

In comparison to laboratory animals the spermatogenetic efficiency in man appears to be poor. In this respect one value of interest is the mean elongate spermatid-Sertoli

cell ratio being 3–4 for the human germinal epithelium [26], versus 12 in rats. Even under these conditions the daily rate of spermatozoa production in man is calculated as 3–4 millions per gram of testicular tissue. Based on these calculations a higher number of spermatozoa in the ejaculate should be expected than the rather low value of 20 millions of spermatozoa/ml ejaculate, as suggested by the WHO manual [27] as normal value.

In recent years reports have been published, indicating a decline of spermatozoal concentrations in ejaculates of healthy males during the last decades [28]. It is thought that interfering prenatal influences to the embryonal development of male gonads occur, e.g. by hormones and their metabolites in the drinking water and other nutriments of the mother.

Regulation of spermatogenesis

The process of spermatogenesis in the seminiferous tubules is maintained by different internal and external influences.

Intrinsic regulation

The Leydig cells in the intertubular space secrete testosterone and additional neuroendocrine substances and growth factors. These hormones, transmitters and growth factors are directed to neighbouring Leydig cells, to blood vessels, to the lamina propria of the seminiferous tubules and to Sertoli cells (Fig. 8). They are involved in maintenance of the trophic of Sertoli cells and the cells of peritubular tissue; they influence the contractility of myofibroblasts and in that way regulate the peristaltic movements of seminiferous tubules and the transport of spermatozoa. They also contribute to the regulation of blood flow in the intertubular microvasculature [29].

Furthermore, different growth factors (IGF1, TGFβ, NGF) are delivered from Sertoli cells and several types of germ cells and take part in a complicate circle of regulation of cell functions and developmental processes of germ cells. All factors together represent an independent intratesticular regulation of spermatogenesis. This very intricate system has been investigated mainly in laboratory animals and is still less understood in human.

Extrinsic influences

The local regulation of spermatogenesis in the testis requires the well known extratesticular stimuli provided by the hypothalamus and hypophysis. Pulsatile secretion of gonadotropin releasing hormone (GnRH) of the hypothalamus initiates the release of luteinizing hormone (LH) of the hypophysis. As a result of this stimulus Leydig cells produce testosterone. Testosterone influences not only spermatogenesis in the seminiferous tubules of the testis but is also distributed throughout the body and provides feedback to the hypophysis related to the secretory activity of Leydig cells. Stimulation of Sertoli cells by the pituitary follicle stimulating hormone (FSH) is necessary for the maturation of germ cells. The Sertoli cells itself secrete inhibin in the feedback mechanism directed to the hypophysis. The extratesticular influences are a necessary basis for the function of intratesticular regulations.

Disturbances of spermatogenesis

Proliferation and differentiation of the male germ cells and the intratesticular and extratesticular mechanisms of regulation of spermatogenesis can be disturbed at every level [9]. This may occur as a result of environmental influences or may be due to diseases that directly or indirectly affect spermatogenesis [30,31]. In addition, different nutrive substances, therapeutics, drugs, hormones and their metabolites, different toxic substances or x-radiation may reduce or destroy spermatogenesis. Finally, also a rather simple noxe as increased temperature reduces the spermatogenetic activity of the testis.

Under these negative influences the testis answer rather monotonuous by reduction of spermatogenesis. This may be expressed in the reduced number of mature spermatids, in malformation of spermatids, missing spermiation, disturbance of meiosis, arrest of spermatogenesis at the stage of primary spermatocytes, reduced multiplication or apoptosis of spermatogonia. If spermatogonia survive then spermatogenesis may be rescued. Otherwise spermatogenesis ceases and shadows of seminiferous tubules remain.

Disturbances of spermatogenesis are evaluated in histological sections of testicular biopsies. The most suitable technique is semithin sectioning of epoxy resin embedded material. In semithin section all details of the cells of the testis can be evaluated optimally because of their excellent preservation. Results of histological evaluation of testicular tissue are given in a score count:

Score count for the evaluation of spermatogenesis (modified from Ref. [32])

10 Intact spermatogenesis: many mature spermatids and zones of spermiation

9 modest reduced spermatogenesis: reduced number of mature spermatids, a few zones of spermiation

8 distinct reduced spermatogenesis: few mature spermatids, no spermiation

7 considerably reduced spermatogenesis: no mature spermatids, only immature spermatids, no spermiation

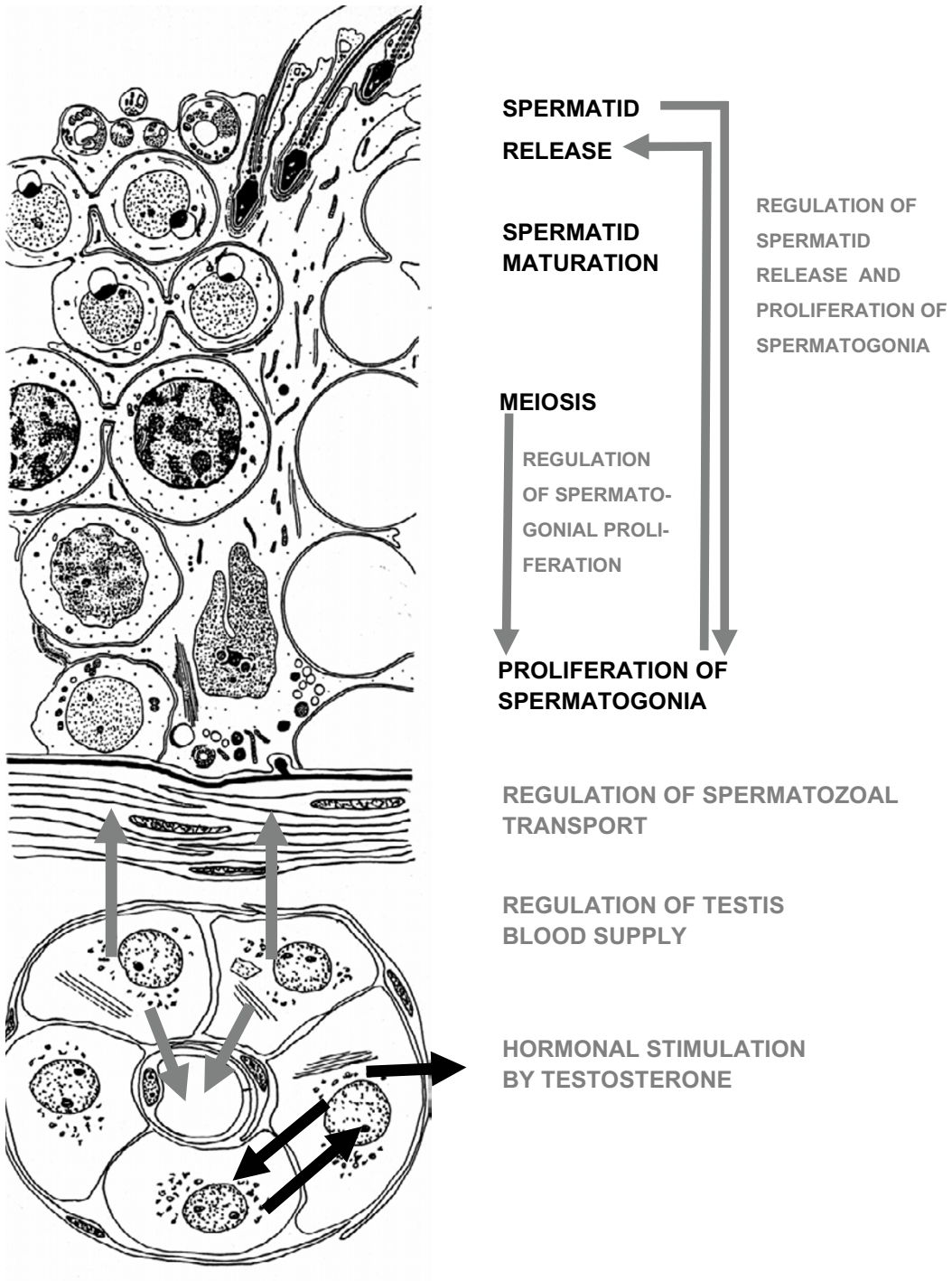

Figure 8
Intrinsic regulation of gametogensis. On the left a section of the germinal epithelium with basal lamina propria and below a cluster of Leydig cells surrounding a capillary are outlined. Arrows indicate different influences of secreted hormones and growth factors. On the right the main processes of spermatogenesis are correlated to regulatory processes.

6 severely reduced spermatogenesis: only few immature spermatids, reduced height of germinal epithelium

5 arrest of spermatogenesis at the stage of primary spermatocytes: many spermatocytes border the lumen of the seminiferous tubule

4 arrest of spermatogenesis at the stage of primary spermatocytes: a few primary spermatocytes are present

3 arrest at the stage of spermatogonia: A type spermatogonia multiplicate but do not develop to maturing cells of spermatogenesis

2 no germ cells, only Sertoli cells are present

1 no germ cells, no Sertoli cells. The seminiferous tubule is replaced by connective tissue ground substance (shadow of tubule)

Understanding spermatogenesis under the aspect of assisted reproduction

Nowadays availble methods of assisted reproduction are founded in basic knowledge of spermatogenesis [33]. By means of specialized techniques of extraction of spermatozoa from testicular tissue (TESE) in combination with intraovocytoplasmatic injection of spermatozoa (ICSI) pregnancies could be induced. Even in deleterious cases of male infertility (azoospermia in the ejaculate, high levels of FSH) in more than 50% ICSI-adapted male gametes could be detected [34] and used for fertilization. The identification of single mature spermatids and spermatozoa is ascertained by means of detailed morphological techniques.

Finally, unrelated these sufficient results it is remarkable that in 0.8 % of the cases under study early testis cancer could be revealed [34].

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