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Glycohistochemical, Immunohistochemical and Ultrastructural Studies of the Bovine Epididymis

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To
My Parents, Wife and Kids

Contents

1 INTRODUCTION

Testicular sperms lack the ability to move and to fertilize an ovum. However, they acquire these properties during their passage through the epididymis (Bedford, 1975). Metamorphosis of an immature and immotile sperm into a mature sperm capable of progressive motility and fertility may be a result of highly regulated and complex sequential events in the epididymis (Cornwall and Hann, 1995). Furthermore, spermatozoa of scrotal mammals possess limited biosynthetic activities (Dacheux et al., 1989) and display a crucial dependence on the activity of the epididymal epithelium for their maturation and survival (Moore and Bedford, 1979, Hinton and Palladino, 1995). The biochemical composition of the epididymal fluid microenvironment exhibits regional variation along the length of the epididymal duct.

This variation results from the differential absorptive and secretory activities of the epididymal epithelium (Carbo, 1960; Levine and Marsh, 1971; Hinton and Palladino, 1995).

The traditional macroscopic segmentation of the epididymis into head, body and tail becomes insufficient to reflect the structural-functional relationship (Glover and Nicander, 1971).

On the basis of histological and histochemical characteristics, the bovine epididymis was subdivided into six segments (Nicander, 1958). Histochemical (Erkmann, 1971; Sinowatz, 1981) and ultrastructural (Sinowatz, 1981) studies confirmed this system of segmentation.

In the present work we have used lectin histochemistry and immunohistochemistry as well as electron microscopic studies to help elucidate the morphofunctional relevance of this scheme of epididymal segmentation and to highlight segment-specific roles of different epididymal regions in maturation of spermatozoa.

2 REVIEW OF LITERATURE

2.1 Development of the Epididymis

2.1.1 Prenatal Development

The epididymis arises from the mesonephric duct, which represents the main excretory way of the primitive excretory system. The pronephric duct arises from the caudal domain of the intermediate mesoderm (nephrogenic mesoderm). The latter, firstly differentiates to form the simplest excretory array (pronephros) in the presumptive neck region (Dyce, et al., 1996). The evolution of the kidney passes through three phases namely pronephros, mesonephros and metanephros, which develop in sequence from cranial to caudal along the urogenital ridge. The pronephros is formed from 7 to 8 pairs of pronephric tubules at the level of somites 7-14. The pronephric duct develops in the intermediate mesoderm of the same region and grows caudally to cloaca. Apart from sheep, pronephric tubules are non-functional and do not establish a patent connection with the pronephric duct. Thus the pronephros is rudimentary in mammals. Caudal to the pronephros and at approximately the level of 9^{th} through 26^{th} somites, 70-80 pairs of mesonephric tubules develop. These tubules are temporarily functional and their formation is initiated by the existence of the pronephric duct. Therefore, they apposed on one end to a blood vessel and to the other they connect into the corresponding caudal extension of the pronephric duct, which is now called the mesonephric duct (Noden and de Lahunta, 1985). The mesonephros is then supplanted by a more efficient excretory system, metanephros, whereas the mesonephros regresses in a craniocaudal direction leaving the most caudal portion of the mesonephric duct and the tubules joining it to perform a similar task but within the male genital system. The metanephros develops at the level of the 26^{th} through 28^{th} somites (Arey, 1965; Noden and de Lahunta, 1985; Günther, 1995; Dyce, et al., 1996).

The chromosomal sex of an embryo is determined at the moment of fertilization. However, the embryos of both sexes, at early stages, pass through a common indifferent stage. In both sexes, the gonadal primordium appears as a thickening of the coelomic epithelium on the medial aspect of the middle part of each mesonephros. It projects as a swelling, as a result of the proliferation of the underlying mesenchyme. This swelling is called the gonadal (genital, urogenital) ridge and initially forms in embryos of approximately 9-10 mm CRL and quickly enlarges due to local hypertrophy of the coelomic epithelium and arrival of the primordial

(primary) germ cells (PGC). The development of the gonadal primordium is coincided and closely linked with the partial degeneration of the mesonephros. In mammals the PGC can first be recognized in the caudal yolk sac endoderm and adjacent to splanchnic mesoderm at about the time of the beginning of somitogenesis. The PGC are then shifted upwards to the dorsal mesentery of the hindgut and then to the mesonephros, which is the site of gonad formation. In cattle, after the breakdown of giant mesonephric corpuscle (GMC), the epithelial cells of the glomerular capsule colonize the gonadal ridge forming the primary sex cords. At this moment the gonad is described as the indifferent stage. The subsequent development of the male gonad takes place in the medullary region of the gonadal ridge.

The gonadal cords form solid tubes that postnatally become patent and form the seminiferous tubules. The gonadal cords are separated from the coelomic epithelium by the primordial tunica albuginea, which is a distinct mesenchymal sheet serving as the pathway of gonadal blood supply. The appearance of the tunica albuginea is the first histological evidence that the gonad is a testis. In the testis the PGC become the spermatogonia that line the seminiferous tubules in association with Sertoli cells, which are derived from the epithelial cells of the mesonephric tubules. The interstitial (Leydig) cells between the seminiferous tubules originate from the mesenchymal cells that originally occupied the gonadal ridge (Arey, 1965; Noden and de Lahunta, 1985). The cords increase in size and in complexity of arrangement. They are arranged in loops that connect via a network of ductules: the rete testis within the testis. The network joins the blind ends of the few mesonephric tubules that have survived the general regression of the mesonephric array. The surviving mesonephric tubules are destined to be the efferent ductules (ED).

At a later stage, the cords are canalized creating a series of tubules, where the peripheral portions become seminiferous tubules and the central portions become the rete testis that joins the ED leading to the mesonephric duct. The latter convolutes within a dense connective tissue forming the epididymis; the remaining part of the mesonephric duct takes a straighter course and continues as deferent duct (Noden and de Lahunta, 1985; Günther, 1995; Dyce, et al., 1996).

The mesonephros in bovine foetus is already maximally developed in the earliest embryonic stages (49 days of gestation). The mesonephric tubules are lined with large truncated columnar cells and gradually degenerate after 49 days, beginning at the anterior end of the kidney in a craniocaudal direction. The mesonephric tubules are converted into efferent ductules between 70 and 150 days, where they show an irregular cuboidal epithelium girdled by a prominent basement membrane and a thin layer of smooth muscle cells (Moustafa and

Hafez, 1971). The ductules develop well and coil at the fourth month of gestation forming the coni vasculosi in caput epididymis. Their epithelium contains ciliated and nonciliated cells. The mesonephric duct gives rise to the epididymis and the deferent duct. Epididymal duct develops from the portion of the mesonephric duct where the caudal ED opens. The epididymis progresses and takes its way to develop in the body of bovine foetus (60^{th} pcd) and to coil (110^{th} pcd; Rüsse and Sinowatz, 1991). After degeneration of mesonephros the epididymis begins to lengthen and convolute strongly at the fourth month of gestation. The convoluted duct forms the caput, corpus and cauda epididymidis. The caput epididymidis forms mainly of ED and the initial segment of the epididymal duct, corpus epididymidis runs laterally along the gonad and cauda epididymidis continues as ductus deferens (Rüsse and Sinowatz, 1991). The epithelium lining the epididymal duct was firstly simple cuboidal to low columnar, which differentiates later on to the characteristic slender high columnar cells with stereocilia (Rüsse and Sinowatz, 1991). However, the epithelium lining the epididymal duct in the caput region contains tall columnar cells tufted with stereocilia and their apical cytoplasm housed secretory granules and droplets, which are first evident at 150 days of gestation (Moustafa and Hafez, 1971). A thin layer of smooth muscle cells surrounds the epithelium of the entire epididymal duct; the thickness of this layer increases distalwards (Moustafa and Hafez, 1971; Rüsse and Sinowatz, 1991).

2.1.2 Postnatal Development

The postnatal development of the ovine epididymis was studied by Carmon and Green (1952). The epididymal epithelium of 6-week aged male lambs showed some stratification. At the 10^{th} week of age, the epithelium nearly attains its mature development. In male goats up to 10^{th} week of age, the epididymis is lined by a single layer of epithelial cells and no stereocilia were observed. After 10 weeks, the epithelium acquired the stratification and became of the pseudostratified type (Yao and Eaton, 1954). Despite the variability in height of the epididymal epithelium in both newborn calves and adult bulls, the epithelium of the newborns was simple in all regions with the exception of the distal portion of the cauda. Differentiation from columnar epithelium to pseudostratified is due to the appearance of the basal cells, which are probably developed from the columnar cells. Two types of cell division take place in the epididymal epithelium of the newborn calves. In the first type, the nucleus moves towards the free border of the epithelium and results in two new columnar cells, whereas the second type occurs near the basal lamina giving rise to the basal cells (Abd El-Raouf, 1960).

The differentiation of the epithelium of the caput, corpus and proximal part of the cauda epididymidis is completed before the epithelium attained its adult height. The distal part of the cauda epididymidis is already differentiated in newborns and the postnatal change in this region mainly concerns the height of the epithelium. The differentiation process is found to be completed in all the different regions at about the age of 32 weeks, while the increase in height continues until the age of 48 to 52 weeks. Both processes follow a similar pattern. They begin in the cauda epididymidis and progress in an ascending manner (Abd El-Raouf, 1960).

2.2 Macroscopical anatomy and species differences

2.2.1 General view

The epididymis is a firm organ that is largely formed by the numerous convolutions of the single epididymal duct within a connective tissue matrix. The epididymis is conventionally divided into three regions, namely head, body and tail (Nickel et al., 1999). This arbitrary division is not corresponding to the functional distinctions (Dyce et al., 1996).

2.2.2 Species differences

The canine epididymis is comparatively large and closely attached along the dorsal part of the lateral surface of the horizontally located testis. It is connected with the margo epididymalis of the testis through the mesoepididymis. Macroscopically, the canine epididymis can be also divided into head, body, and tail. The latter is connected with the posterior extremity of the testis through the ligamentum testis proprium, and with the vaginal process through the ligamentum caudae epididymidis (Orsi, 1983; Nickel et al., 1999).

The equine epididymis consists of three grossly distinct regions and the entire length of the ductus epididymidis ranges from 70 to 90 meter (Ghetie, 1939). Because of the horizontal orientation of the testicular long axes, the epididymal portions are described as cranially located, enlarged head, intermediate narrow body, and the caudally located, slightly enlarged tail (Nickel et al., 1999). The head is closely attached to the testis by the efferent ductules, by the connective tissue and by the serous membrane. The head consists of a twelve or more coiled efferent tubules, which are grouped into lobules. Each of the latter consists of four to five tubules, which unite to form a single tube. They join with the tube of other lobules and into the single wider epididymal duct (Sisson and Grossman, 1943). The latter forms many convolutions, which are held together by connective tissue and smooth muscle fibres

forming parts of the head, the body and the tail of the epididymis; terminates in the ductus deferens. The body is less closely attached by the serous covering leaving a lateral pocket (sinus epididymidis) beneath the epididymis. The tail is continued by the ductus deferens and is attached to the posterior extremity of the testis by the epididymal ligament (Nickel et al., 1999).

The caprine epididymis is a long, highly convoluted duct, which forms its head, body and tail. The head rests on the dorsocaudal border of the testis; the body is represented by a thin strip-like structure located on the caudomedial border of the testis. The tail is an enlarged promontory-like structure located on the ventromedial border of the testis and is easily palpable through the scrotum in mature goats. The epididymal head consists of a descending and an ascending limb. Both limbs can be easily detached from the underlying testis, by blunt dissection, except at the papilla where the extratesticular rete testis enters the bulbous portion of the descending limb. This bulbous portion is characterized by its brownish appearance and contains, at its most proximal end, the so-called appendix epididymidis. The main components of this portion are efferent ductules, a few ductules of paradidymidis within the connective tissue separating the lobules of the efferent ductules, and parts of the extra-testicular rete testis. The remaining two thirds of the descending limb and the ascending limb are arranged in pale-coloured small lobules, except at the junction of the two limbs where one or two lobules always have a brownish appearance (Goyal and Williams, 1991).

The bovine ductus epididymidis is 40 to 50 meter long (Sisson and Grossman, 1943) and the epididymis is attached along the caudomedial border of the testis. It usually spreads some distance over both proximal (caput epididymidis) and distal (cauda epididymidis) poles of the testis (Dyce et al., 1996). The epididymal head is firmly attached to the testicular capsule receiving the efferent ductules, which join to form a wider single epididymal duct (Nickel et al., 1999). The body may be less completely attached to the testicular surface leaving an intervening space (testicular bursa) in-between. The tail is firmly attached to the testis by a ligament (proper ligament of the testis) and to the parietal layer of the tunica vaginalis by the ligament of cauda epididymidis. The tail finally tapers and emerges as ductus deferens (Dyce et al., 1996). The bovine epididymis can macroscopically be divided into three main segments: the head, the body, and the tail. The epididymal head consists of a descending and an ascending limb. The former is composed of three functionally active parts: extratesticular rete testis, efferent ductules and epididymal duct (Tröger, 1969; Goyal, 1983), and two presumably non-functional parts: the appendix epididymidis and aberrant ductules (Goyal,

1983, 1985). The extratesticular rete testis is short and located between the dorsal extremity of the testis and ventromedial side of the efferent ductules. The latter forms a brownish bulbous structure at the proximal end of the descending limb and is located on the fatty tissue covering the base of the spermatic cord. The epididymal component of both descending and ascending limb is cream in colour except at the junction of two limbs, where it has a brown colour (segment II). The appendix epididymidis is cream in colour and occupies the most proximal part of the descending limb. The aberrant ductules cannot be recognized macroscopically but can be easily seen with the light microscope. These ductules consists of two to four small groups of tubular cross sections located in the connective tissue between the lobules of the efferent ductules, and in the connective tissue separating the efferent ductules from the initial segment of the epididymis. The epididymal body is narrow and attached to the lateral side of the caudal border of the testis. The epididymal tail is large, attached on the ventral extremity of the testis, and can be easily palpated through the scrotum (Goyal, 1983, 1985).

The number of the efferent ductules of different species (Hemeida et al., 1978) is summarized in table 1. Opposite to the bovine epididymal head, rete testis penetrates the tunica albuginea and extends cranioventrally, closely applied to the tunic for 8-12mm (extra-testicular rete), before giving rise to 13-16 efferent ductules. Typically, ductules 1-4 join, and the epididymal duct begins 8-10 mm distal to this junction. The remaining ductules join the duct singly or in pairs through relatively long (12-31 mm) connecting ductules. An efferent ductule may be divided into three segments: a slight undulating testicular segment (A), a highly coiled cone segment (B) and a moderately coiled thinner epididymal segment (C). Adjacent ductules, which join before ending at the epididymal duct, continue as connecting ductules. They have the same appearance as segment C and provide the connection with the epididymal duct (Hemeida et al., 1978).

2.2.3 Blood supply of the epididymis

The vascularisation of the bovine epididymis was thoroughly studied by Clavert (1980) and Amselgruber (1988). The epididymis gets a dual arterial blood supply, from the internal spermatic artery supplying the head and the body, and from the deferential artery, which supplies mainly the epididymal tail (Gunn and Gould, 1975). The composition of blood supplying the head and the body of the epididymis may differ from that arriving from the systemic circulation because of the possibility of counter-current movement of substances between the spermatic veins and artery within the pampiniform plexus. Androgens are found

in a high concentration in the testicular venous return (Free, 1976; Free and Tilson, 1976). Anastomoses between veins and artery have been established in bulls (Hees et al., 1984) and rams (Noordhuizen-Stassen et al., 1985) near the branching of the epididymal artery and this may be involved in the reflux even of large molecules into the arterial circulation (Cooper, 1986).

A mesh of vessels surrounding the duct system like a fishnet stocking represents the microvasculature of the bovine ED and epididymal duct. Both the "zwickel capillaries" and the subepithelially located capillaries are of the continuous type. Both of the initial portions of the ED and the sixth segment (cauda epididymidis) possess a particularly high capillary density. The increased capillary density seen in the initial portion of the ED may be related to their secretory and resorptive activities. The unusual dense, two-layered configuration of capillaries under the lamina epithelialis of the bovine cauda epididymidis provides a sound morphological basis for intensive metabolic processes (Amselgruber and Sinowatz, 1990).

The microvasculature of the epididymis has been also studied in rats (Kormano, 1968), mice (Suzuki, 1982) and rabbits (Clavert et al., 1981). The density of the capillary network is highest in the initial segment of the epididymis in mice (Takano, 1980) and rabbit (Clavert et al., 1981). The density of the capillary network in the initial epididymal segment is decreased after androgen deprivation following bilateral castration (Clavert et al., 1981).

Table 1: the ranges of the numbers of ED in different species (Hemeida et al., 1978)

Animal species	**Number of ED (range)**
Dog	13-15
Cat	14-17
Boar	14-16
Goat	18-19
Ram	17-20
Bull	13-16
Stallion	14-17

2.2.4 Lymphatic drainage

Major lymphatic vessels drain the head, the body and the tail of the epididymis of rabbits (Clavert, 1981) and rats (Pérez-Clavier et al., 1982). There are wide intertubular lymphatic sinusoids in the mouse (Suzuki, 1982) and rat (Kazeem, 1983). Furthermore, the

spermatozoa have been found in the regional lymph nodes in boars and rams following vasectomy (Ball and Setchell, 1983), pointing to a process of spermatophagy as a result of breakdown of epididymal barrier.

2.2.5 Nerve supply

The epididymis is under the control of autonomic nervous system. The vegetative nerve plexus arise from the intermesenteric, rather from the caudal mesenteric plexus. The testicular nerve plexus supplies the testicular artery, testis, and epididymis. The accessory ganglia can be found at the origin of the testicular artery or along the course of the nerve plexus. The epididymal nerves are also found interepithelially (El Badwi and Schenk, 1967) and axons can be next to blood vessels and epithelial basal membrane. The epididymis receives its parasympathetic supply from the caudal mesenteric and testicular plexus, which get participation from the dorsal vagal trunk and branches of intermesentric plexus.

2.3 Light Histological Structure of Mammalian Epididymis

2.3.1 General view

The mammalian epididymis consists of a single ductus epididymidis and several ductuli efferentes, which connect the rete testis to the ductus epididymidis (Patt and Patt, 1969; Aughey and Frye, 2001). Macroscopically, the epididymis is divided into a head, body and tail. It is encircled by a thick tunica albuginea of dense irregular connective tissue covered by the visceral layer of tunica vaginalis (Patt and Patt, 1969; Wrobel, 1998). The initial portion of the ductus epididymidis together with the ductuli efferentes constitutes the head of the epididymis (Patt and Patt, 1969).

The ductuli efferentes (from 12 to 25 in number) originate from the rete testis and are arranged in such a way that they look like as a cone with the tip pointing towards the rete testis (Wrobel, 1998). The ductuli efferentes are lined by ciliated pseudostratified columnar epithelium. The nonciliated cells carry microvilli. In addition to ciliated and nonciliated, occasional basal cells are present (Patt and Patt, 1969; Wrobel, 1998; Aughey and Frye, 2001).

The epithelium of efferent ductules (ED) in all species contains two major cell types, ciliated and nonciliated cells; and a few intraepithelial lymphocytes and macrophages (Robaire and Hermo, 1988; Ilio and Hess, 1994). The epithelium of ED of the macaque monkey features primarily ciliated and nonciliated cells. Occasionally intraepithelial lymphocytes (IEL) and

macrophages (IEM) are encountered, but the macrophages were more frequently seen in the lumina and the periductal connective tissues (Ramos and Dym, 1977b).

The epididymal duct is extremely tortuous and coiled tube. It is lined with pseudostratified columnar epithelium with spherical or ovoid lightly stained basal cells and the apical surface of the columnar cells carries long stereocilia. The ductus epididymidis is surrounded by a small amount of loose connective tissue (mainly of reticular fibres) and circularly oriented smooth muscle fibres, the number of which increases gradually toward the tail of the epididymis. This muscle layer inturn is surrounded by loose connective tissue, which occasionally forms somewhat denser and thicker strands and blends with the tunica albuginea (Wrobel, 1998).

According to the distinct macroscopic features, the epididymis was divided into head, body and tail (Nickel et al., 1999). However, this system of segmentation is insufficient to reflect the structural-functional relationship (Glover and Nicander, 1971). Regional differences in the histological structure of the epididymal epithelium have been early recorded in dogs (Hammer, 1897); in rabbits, rats and stallion (Aigner, 1900**)**, and in bulls (Friedrichs, 1906; Redenz, 1924). Further investigations have been performed in different animal species and by the use of various methods in an attempt to prove a structure-function relationship (Glover and Nicander, 1971). Accordingly, the epididymis of most mammalian species may be divided, based on histological and cytochemical characteristics, into an initial segment, a middle segment, and a terminal segment. The first two segments make up the part of the epididymis concerned with sperm maturation, whereas the terminal segment is engaged in sperm storage (Glover and Nicander, 1971). However, these three segments do not correspond to the areas described as head, body and tail of the epididymis, which relate only to macroscopic structure and are used simply for convenience in general orientation (Glover and Nicander, 1971). Furthermore, based on histological, histochemical, and ultrastructural features, the epididymis may be subdivided into several segments, whose distribution and number are characteristic for each species (Wrobel, 1998).

In the following, some specific characteristics for several mammalian species are given:

- The segmentation of the epididymis in rabbits was recorded by Nicander (1957). Constant regional differences, with regard to the width and contents of the lumen, as well as the height, structure and cytochemistry of the epithelium, are evident.

Accordingly, the epididymis is subdivided into eight regions.

- According to the characteristics of the epithelium, the epididymis of the guinea pig (Hoffer and Greenberg, 1978) is subdivided into seven zones each of which could be readily distinguished based on its histological features and substantially on the appearance of the principal cells (PC).
- In cats (Sanchez et al., 1998) and based on the morphological features of the epithelium and its various cellular components, the shape and diameter of the lumen and the thickness of the peritubular muscular coat (PMC), the epididymis is divided into four morphologically different regions.
- The caprine epididymis, based on the histological features and the cytological characteristics of the PC, was subdivided into five segments. The head consists of the regions I, II, and proximal portion of region III. The distal portion of region III together with region IV constitutes the body. The tail is represented by region V. The different regions show distinct variations in the height of the epithelium, luminal diameter, thickness of the PMC, the luminal sperm concentration and the cytological features of the principal cells as well. However, there is no abrupt, but gradual transition between the consecutive regions with the result of a possible overlapping of the morphological characteristics between two adjacent regions (Goyal and Williams, 1991).
- Nicander (1958) has proposed a scheme of segmentation of the epididymis in bulls, rams and stallion, where the epididymal duct was subdivided into six regions in each. This segmentation based on the regional variation of the epithelial height, the position, the size and the form of the nuclei of the PC. This was confirmed later on, for bovine epididymis, by the work of Erkmann (1971), who has applied histochemical means, by Sinowatz (1981) and by Goyal (1985), who have used histochemical and ultrastructural methods.

2.3.2 Light Histological Structure of the Bovine Epididymis

According to the work, which had been begun by Nicander (1958) and has been proved by Erkmann (1971), Sinowatz (1981) and Goyal (1985), the bovine epididymis could be sub-divided into six regions. The first three (I, II, and III) segments constitute the head (caput epididymidis), the following two segments (IV and V) the body (corpus epididymidis), whereas the tail (cauda epididymidis) is represented by the sixth (VI) segments. Epididymal head consists of two limbs, descending and ascending. The former consists of three

functionally active structures: extratesticular rete testis, efferent ductules and the initial portion of the epididymal duct. This initial portion represents the first (I) epididymal segment. The ascending limb constitutes the third (III) epididymal segment, whereas the site of the junction between both limbs constitutes the second (II) segment (Goyal, 1985), which was described by Erkmann (1971) as the knee of the epididymal head.

2.3.2.1 Efferent ductules (ED)

The bovine efferent ductules (ED) are arranged in lobules with indistinct boundaries. Their cross sections are densely packed. The epithelial characteristics are similar in a lobule; however they may be variable in the area between two adjacent lobules (Goyal and Hrudka, 1981). The ED are lined by ciliated pseudostratified columnar epithelium. The epithelium consists of ciliated and nonciliated columnar cells, with occasional basal cells (Wrobel, 1972; Hemeida et al., 1978; Aughey and Frye, 2001). The ciliated cells make up about 80 % of the lining epithelium (Hemeida et al., 1978); however, Goyal and Hrudka (1981) reported that ciliated cells exist in numbers comparable to that of the nonciliated ones. They are distinguished not only by the cilia but also by the position of the nucleus. The nuclei are ovoid and heterochromatic and located near the luminal end of the cell (Goyal and Hrudka, 1981). The luminal surface of the cell is provided by kinocilia with a few microvilli scattered in-betweens. IEL are found scattered throughout the epithelium and are characterized by a strongly heterochromatic nucleus. IEM with pseudopodia are also seen (Goyal and Hrudka, 1981).

The nonciliated cells are classified according to their cytological features into three types: type I cells contain neither granules nor vacuoles, type II cells possess granules, and type III cells have vacuoles. Luminal surfaces of all the three cell types are provided with microvilli, apical protrusions or both of them. The apical protrusions may contain granules or vacuoles. They are also found separated in the lumen, along with granules and vacuoles. The nuclei of all the three cell types are oval, moderately basophilic to euchromatic and located in the basal third of the cell (Goyal and Hrudka, 1981). The specific granules of the type II cells stain with eosin and PAS but are negative after Alcian blue. This suggests that the content of the granules is a glycoprotein. The granules are, in general, round and mostly homogenous. They seem to fill the entire cytoplasm and increase in size from basal to luminal pole of the cell. However, variation in their size, shape, density and number are not uncommon among cells or within a cell (Goyal and Hrudka, 1981). The vacuoles of the type III cells do not stain with eosin, PAS or Alcian blue. The flocculent and membranous content of the

vacuoles may indicate low-density phospholipids. The vacuoles exist in the supranuclear half of the cell, but unlike the granules they were never seen in the infranuclear area. They were often arranged in stacks in a peg-and-socket manner. The vacuoles disappear after orchidectomy (Goyal and Hrudka, 1981).

In addition to the three typical nonciliated cell types, another profile of cells is occasionally encountered. These cells contained both granules and vacuoles. However, unlike those of type II cells, the granules are smaller, less dense and confined to the intermediate supranuclear region. The vacuoles occupy the remaining apical cytoplasm and arranged in a peg-and-socket array. Similar to the other cell types, the luminal surface was modified to form a microvillous border or an apical protrusion. Owing to the rare occurrence of this cell type and its restriction to only the middle area of the efferent ductules, they are described as transitional or aberrant type of cells (Goyal and Hrudka, 1981).

The distribution of all the three cell types of nonciliated cells was determined. The ED are divided into proximal, middle and distal areas. Type I cells is found throughout the entire length of the efferent ductules. Type II cells predominate in the proximal area whereas type III cells predominate in the distal area of the ED. However, simultaneous occurrence of both types II and III cells in the middle area may account for the overlap of segments (Goyal and Hrudka, 1981).

2.3.2.2 Epididymal duct

2.3.2.2.1 Segment I

This region is characterized by a variable epithelial height within every cross-section especially in the proximal portion of the region (Nicander, 1958). The lumina of the crossly cut tubules exhibit a triangular to stellate appearance. This is the result of the irregular height (65-120 μm) of the epithelium (Sinowatz, 1981). In old bulls, some crossly cut tubules reveal intraepithelial glands (Nicander, 1958), which were described later on by Tröger (1969), Erkmann (1971) and Sinowatz (1981) as deep epithelial crypts. The lumina usually contain few numbers (Nicander, 1957; Sinowatz, 1981) or absolutely no spermatozoa (Erkmann, 1971). The tubules are lined by pseudostratified epithelium of mainly two cell types, tall slender principal cells (PC) and polygonal basal cells (BC) as well as intra-epithelial leukocytes (Nicander, 1958; Erkmann, 1971; Sinowatz, 1981; Goyal, 1985 and Wrobel, 1998). The PC are the main cell type, which have variable height, and their apical surface is provided by stereocilia of about 15 μm long (Nicander, 1958; Erkmann, 1971) and

possesses lightly stained vacuolar structures in the supranuclear cytoplasm (Goyal, 1985). The infranuclear cytoplasm contains many small vacuoles, which are faintly stained with PAS (Nicander, 1958; Goyal, 1985) and sometimes similar granules in the supranuclear cytoplasm, especially in older animals (Nicander, 1958). The nuclei of the PC have oval to elongated form with two to three distinct nucleoli and are located close to the basal lamina (Nicander, 1958; Goyal, 1985) or in the basal third of the cell (Erkmann, 1971). However, a few nuclei of the PC are also seen near the apical area of the epithelium and occasionally showed mitotic figures (Goyal, 1985). The nuclei reach a size of about 13 µm (Nicander, 1958).

The second cell type is the small (7 µm) polygonal basal cells (BC), which are characterized by a higher nuclear: cytoplasmic ratio and pyramidal to kidney-shaped nuclei with deep invaginations (Sinowatz, 1981; Goyal, 1985) which are surrounded by a narrow cytoplasmic rim (Erkmann, 1971). This type of cells rest on the basal membrane and never reach the luminal surface and exists sparsely in this region.

The cells, which were early described as halo cells, have been found here with heterochromatic round nuclei and scanty lightly stained cytoplasm. These cells have been proved to be intraepithelial lymphocytes (IEL) and represent the third cell type (Sinowatz, 1981; Goyal, 1985). The intraepithelial macrophages (IEM) are the fourth cell type, which are usually found near the basal lamina. These cells have eccentrically located nuclei and strongly PAS-positive granules (Erkmann, 1971; Goyal, 1985). The intertubular connective tissue contains macrophages, lymphocytes, plasma cells and mast cells (Sinowatz, 1981; Goyal, 1985). The peritubular muscular coat (PMC) is formed of 5 to 7 smooth muscle cell layers of about 15 to 20 µm thick (Lindinger-Niederhofer, 1991).

2.3.2.2.2 Segment II

The epithelial height is lower (about 70 µm) and the lumen is more regular than that of the first segment. The nuclei are short ovoids or even spheres and form a multi-layered nuclear zone in the basal area of the epithelium, without a line of demarcation between the nuclei of PC and those of BC (Nicander, 1958; Erkmann, 1971; Sinowatz, 1981). In addition to the cell types that exist in the first segment, there are, in some cross sections, the apical mitochondria-rich cells (AMRC), which occur only in low numbers (Sinowatz, 1981). These cells are characterized by their apical location, their narrow body, pale cytoplasm, and a dome-shaped luminal border with a few short microvilli. The nuclei of these cells are round to oval and present in the apical third of the cell (Sinowatz, 1981; Goyal, 1985). The IEL and

IEM were also seen as in the segment I. The PMC is formed of 3 to 4 layers of smooth muscle cells and its thickness reaches about 20 µm (Lindinger-Niederhofer, 1991).

2.3.2.2.3 Segment III

The epithelium becomes higher (80 µm) again and the nuclei are elongated and located at a higher level in the cytoplasm than previous. The outlines of the nuclei are often slightly irregular (Nicander, 1958; Erkmann, 1971; Sinowatz, 1981). The lumina are regular round to slightly oval and are studded with spermatozoa (Nicander, 1958; Erkmann, 1971; Sinowatz, 1981), as well as various types of cells with round nuclei, which probably originate from germinal epithelium (Sinowatz, 1981). The luminal surface of the PC is provided by 10 µm long stereocilia (Sinowatz, 1981). Not only the number but also the size of the BC has increased compared to the former two segments (Nicander, 1958). Similarly, the number of IEM increased in comparison to that of the first two segments. They are mostly found in the basal area and only sporadically in the apical area (Sinowatz, 1981). Both of AMRC and IEL are present in numbers and appearance similar to that encountered before. The thickness of the PMC is about 30µm (Lindinger-Niederhofer, 1991).

2.3.2.2.4 Segment IV

The lumina are densely filled with spermatozoa (Nicander, 1958). Most of the crossly cut tubules exhibit round to slightly oval contour; however, in sporadic cases slight folding was encountered (Erkmann, 1971). The epithelium is somewhat higher (80 µm) than that of the third segment (Sinowatz, 1981; Goyal, 1985). On the contrary, Nicander (1958) recorded that it is somewhat lower (65 µm) than that of segment III. The nuclei of the PC are vesicular and occupy a wider area in the middle of the cell forming a nuclear zone, which is well-demarcated from the nuclei of the BC. Due to the vertical expansion of the nuclei of PC, a decrease of the supranuclear area than in the preceding regions (Erkmann, 1971). These nuclei are elongated (about 17 µm), slightly irregular and with pointed apices (Nicander, 1958). The PC have a small group of tiny granules occupying the most basal cytoplasm and stain faintly with PAS (Nicander, 1958). The BC are somewhat more numerous (Nicander, 1958; Sinowatz, 1981), whereas the numbers of IEL and IEM are diminished (Sinowatz, 1981). Only very few AMRC are seen (Sinowatz, 1981; Goyal, 1985). The nuclei of the BC have in general a dark appearance (Erkmann, 1971). The thickness of the PMC increases and reaches 35 µm (Lindinger-Niederhofer, 1991).

2.3.2.2.5 Segment V

The epithelium of this region is taller than that of regions II-IV (Goyal, 1985). The fifth segment can hardly be differentiated from the fourth one (Nicander, 1958; Erkmann, 1971). The main difference is represented by the well-distinct epithelial folds, which make the luminal surface wavy, with tops or combs (up to 100 μm epithelial height) and bottoms or valleys (up to 70 μm epithelial height). Thus, the contour of the lumen is often stellate-shaped (Sinowatz, 1981). The morphology of the PC is the same of segment IV and their nuclei are narrow elongated and generally located in the middle of the cell (Erkmann, 1971) forming a narrower nuclear zone (Nicander, 1958). The BC appear to be more numerous than in any other segment of the epididymis (Nicander, 1958; Goyal, 1985) and form a dense cell layer (Erkmann, 1971). The PMC is about 40 to 45 μm and is formed of 6 to 9 smooth muscle cell layers (Lindinger-Niederhofer, 1991).

2.3.2.2.6 Segment VI

This region is characterized by the very wide lumina, which are surrounded by a somewhat regular epithelium. They are densely crowded with spermatozoa (Erkmann, 1971). The proximal portion of this region has folded epithelium; the epithelial folds fade downwards in the direction of ductus deferens (Sinowatz, 1981). On the other hand, Erkmann (1971) recorded that the epithelium becomes continually folded. Contrary to the conditions in most species, the epithelium is still high (60μm) in this region (Nicander, 1958), but the stereocilia are lower (5 to 8 μm) as recorded by Nicander (1958) and Sinowatz (1981). This region is characterized by lower epithelium, greater luminal diameter, greater sperm concentration, denser and shorter stereocilia and a thicker PMC than in any other region of the epididymis (Goyal, 1985). The nuclei of the PC have irregular outline and are deeply invaginated (Nicander, 1958; Goyal, 1985) and in some cells appear almost lobated (Sinowatz, 1981; Goyal, 1985). The nuclei of the PC are somewhat displaced towards the basal membrane (Erkmann, 1971). The nuclei of the BC constitute a loose layer with the cells somewhat larger than in any of other segment of the epididymis (Erkmann, 1971). The AMRC are not found (Goyal, 1985). The IEM and IEL exist in variable number (Sinowatz, 1981). BC present the same morphology as described for the previous regions (Goyal, 1985). The sixth segment is characterized by a well-distinct PMC and its thickness reaches about 80 μm and is formed by about 10 smooth muscle cell layers (Lindinger-Niederhofer, 1991).

2.4 Lectin histochemistry

2.4.1 Definition of a lectin

The term lectin (Latin: legere = to choose; to select) was first used to describe a group of plant seed agglutinins; some of them were specific for human blood groups (Boyd and Shapleigh 1954). A lectin is a carbohydrate-binding protein of non-immune origin which has the capability to agglutinate cells (Goldstein et al., 1980). Lectins are proteins that specifically recognize sugar residues and when they are conjugated with a chromogen, they can be used to localize carbohydrate groups in the tissues (Spicer, 1992).

2.4.2 Common structure and molecular properties of lectins

Legume lectins are a large family of homologous proteins of common structural properties, which have been extensively studied for their carbohydrate-binding properties and used in biology and medicine (Lis and Sharon, 1977). They are initially synthesized as single polypeptide chains of a molecular weight of about 30,000 Da. After excluding of a 20-residue hydrophobic sequence, this chain may be post-synthetically cleaved into two subunits: a heavy β-chain and a light α-chain. Structurally, the lectins are either one-chain lectins (of identical or nearly identical subunits of 25,000-30,000 Da) or two-chain lectins consisting of two different subunits, a light α-chain and a heavy β-chain. The subunits of lectins may be associated into dimers of about 50,000 Da or tetramers of 100,000 to 120,000 Da. The *Lima bean* agglutinin is a legume lectin, which has been reported to contain a disulfide bond, which links the two subunits (each of 31,000 Da) of the protein. Non-covalent interactions are also involved in subunit binding of many legume lectins. Owing to variable degrees of glycosylation, many lectins are glycoproteins and this may account for the diverse molecular weights and polymorphism. Based on binding a particular monosaccharide, the lectins of a single plant may be apparently functionally homogenous, thus can be purified by affinity chromatography. However, the electrophoretic analysis of the purified lectins may reveal extensive variations. These variations may be attributed to a complex interaction between genetic polymorphism, post-synthetic modifications (cleavage or glycosylation) and species polymorphism. Owing to the homology between the sequences of the one- and two-chain lectins, they exhibit a close resemblance in their folding and three-dimensional structure (Strosberg et al., 1986). It was proved by computational methods, that legume lectins share common three-dimensional structure (Strosberg et al., 1986). Various

lectins share structural and functional sites, which are principally based on the amino acid sequences. These sites include:

- Metal binding sites: These binding sites are formed of amino acid residues that interact directly with metal ions such as Ca^{++} and Mn^{++}.

- Hydrophobic cavity: This cavity of the three-dimensional structure of lectins is formed by certain sequence of amino acid residues. It is so conserved through the evolution and this supports its essential role in the function of lectins. This site binds glycosides or other hydrophobic sugar derivatives. The hydrophobic region is located near to the carbohydrate binding sites of lectins. Furthermore, several lectins bind hydrophobic compounds devoid of sugar moieties. Such binding is not inhibited by specific sugars indicating that hydrophobic ligands bind to lectins at sites distinct from carbohydrate binding sites.

- Glycosylation sites: Glycosylation is the incorporation of a carbohydrate moiety to be attached to a characteristic sequence of amino acids of a lectin. This sequence is absent in lentil lectin and Concanavalin A. Thus both are non-glycosylated lectins. The site of carbohydrate attachment is not conserved among the glycosylated lectins.

- Carbohydrate binding sites: The amino acid residues that constitute the sugar binding sites are not so conserved that all the members of a certain saccharide-specific lectin group exhibit the same binding-affinity. Generally, there is one sugar-binding site per subunit of a lectin molecule. However, some exceptions exist in many lectins; for example, tetrameric lectins from *Datura stramonium* express only two binding sites. On the other hand, each subunit of Wheat germ agglutinin exhibits two binding sites. The subunits of a certain type of a lectin usually have the same sugar specificity (Lis and Sharon, 1986).

2.4.3 Biological properties of lectins

a) Agglutination:

Hemagglutination was the first recognizable biological activity of lectins. Consequently this property was the most frequent method for detection of lectins using freshly drawn human or animal erythrocytes (Lis and Sharon, 1973; Burger, 1974). The ability of lectins to agglutinate cells, distinguishes them from other sugar-binding macromolecules, such as glycosidases and glycosyltransferases, and is therefore incorporated in the definition of lectins suggested by Goldstein et al. (1980).

b) Mitogenic stimulation of lymphocytes:

Stimulation of mitogenesis of lymphocytes represents one of the most dramatic effects of

the interaction of lectins with cells. The mitogenic stimulatory effect of certain lectins triggers the quiescent, nondividing lymphocytes into a state of growth and proliferation (Lis and Sharon, 1977). The mechanism of mitogenic stimulation still obscure, however, it has been suggested that mitogenic lectins interact with unique membrane components that may act as triggering receptors. The latter may not be recognized by the nonmitogenic lectins (Lis and Sharon, 1986).

c) Induction of suppressor cells:

Suppression of lymphocyte activities could be mediated through the effect of certain lectins. Various mitogens, in particular Concanavalin A, induce a generation of potent suppressor cells capable of inhibiting activities of T- and B-lymphocytes *in vitro* (Haynes and Fauci, 1977; Fineman et al., 1979; Lis and Sharon, 1986). The suppressive effect might be, at least partly, mediated by soluble factors released by suppressor cells (Fleisher et al., 1981; Greene et al., 1981). The suppression of T-lymphocytes is probably a result of the inhibition of antigen- and mitogen-stimulated T-cell proliferation (Greene et al., 1981; Barrett et al., 1983). Suppression of B-lymphocytes could be attributed to inhibition of their immuno-globulin production (Fleisher et al., 1981). The factor, which inhibits the activity of T-cells, binds to at least some of the surface glycoproteins (triggering receptors) to which the mitogenic lectins bind (Greene et al., 1981; Barrett et al., 1983).

d) Lectin-dependent cytotoxicity of lymphocytes and macrophages:

Lectin-dependent cytotoxicity is a phenomenon that takes place, in the presence of mitogenic lectins, where a wide variety of antigenically unrelated cells undergo cytolysis as a result of T-cell mediated cytotoxicity (Asherson et al., 1973). The lack of immune specificity in the lectin-dependent reaction has been attributed to the ability of the lectin to bind both effector and target cells. This promotes proximity between them and facilitates the cytolytic activity of the effector cells (Lis and Sharon, 1986). In addition to bridging effector and target cells, lectins have other significant roles. They can activate effector cells to kill nonspecifically target cells (Parker and Martz, 1980) or modify target cell to express structural alterations essential for cytolysis (Bonavida and Katz, 1985). Similar to anti-tumour antibodies that can induce macrophage-mediated tumour lyses, lectins either of plant origin (Kurisu et al., 1980; Maddox et al., 1982) or insect origin (Ohkuma et al., 1985) have the ability to mediate carbohydrate-specific binding of mouse macrophages and tumour cells and to induce killing of the tumour cells by the macrophages (Lis and Sharon, 1986). It has been suggested that

the lectin-mediated cytotoxicity may occur *in vivo*, since intraperitoneal injection of *Griffonia simplicifolia* agglutinin I (GSA-I) protected mice, inoculated with Ehrlich ascites tumour cells, from tumour growth and subsequent death (Eckhardt et al., 1982; Lis and Sharon, 1986). In addition to lectin-dependent killing of tumour cells by macrophages, lectins mediate binding and, occasionally, phagocytosis of other types of cell (Sharon, 1984).

e) Insulinomimetic activity:

Many lectins, for example Concanavalin A (Con A) and Wheat germ agglutinin (WGA), have insulinomimetic effects on adipocytes, such as stimulation of lipogenesis, glucose transport and oxidation as well as inhibition of lipolysis (Lis and Sharon, 1986). These insulinomimetic activities were observed both *in vitro* (Katzen et al., 1981; Shechter and Sela 1981) and *in vivo* (Katzen et al., 1981).

2.4.4 Lectin toxicity

Several lectins, for example, Con A, WGA and Phytohemagglutinin (PHA) are toxic to mammalian cells both *in vitro* and *in vivo*. However, their toxicity is much less than that of Ricin (Lis and Sharon, 1986). Toxic lectins are generally selective in their action on cells. Transformed cells, in particular, are much more sensitive to cytotoxic effects of lectins than normal cells (Nicolson, 1974). Therefore, several attempts were made to inhibit tumour growth by lectins *in vivo* (Eckhardt et al., 1982; Lis and Sharon, 1986). The cytotoxicity of the plant toxins is dependent upon their molecular characteristics. These toxic lectins (e.g. Ricin) consist of two chains joined by disulphide bonds. The heavier (β) chain has the carbohydrate-binding site, whereas the lighter (α) chain inhibits the protein synthesis in cell-free system, representing the toxic moiety of the molecule. The molecule is active on the cells, only when it is intact. Subsequent to binding to cell-surface sugar residues via the β-chain, the toxins are taken up by the cell where the α-chain inhibits protein synthesis by interfering with peptide chain elongation on polyribosomes.

2.4.5 Classification of lectins

Gallagher (1984) proposed not only a system of lectin classification but also a formula for their nomenclature. The lectins are classified according to their sensitivity to different carbohydrate inhibitors into two groups:

1) Class-I (exolectins):

The reactivity of this group of lectins is principally directed towards a particular monosacch-

aride in the ligand. These lectins are strongly inhibited by low concentrations of the appropriate free sugars or their methyl-glycosides. All the class-I lectins bind to compatible external, non-reducing sugars in complex saccharides so that they may be described as exolectins. Whether the exolectins have a stringent requirement for end-chain sugars or no, they are classified into two sub-classes:

a) Obligate exolectins (Class-I a):

These lectins have a stringent need for a compatible end-chain (terminal) sugar to express their carbohydrate-binding activity. This category includes most of the glucosamine (GlcNAc)-specific and galactosamine (GalNAc)-specific lectins.

b) Facultative exolectins (Class-I b):

These lectins bind to monosaccharides either as internal or external (end-chain sugar) constituents. Examples for this category are Con A and RCA.

2) Class-II (endolectins):

These groups of lectins react only with specific carbohydrate sequences and none of the individual sugars in this sequence plays a predominant role in the binding process. Thereby a potent inhibition of their agglutinating activities is achieved only with specific sugar sequences. For the expression of their carbohydrate-binding properties, they essentially need to recognize one or more internal sugars of the complex oligosaccharides. Thus, they are called endolectins. Whether the oligosaccharide consists of identical sugar units or not, they are classified into two groups:

a) Class-II a (homotypic endolectins):

These lectins show the highest affinity to homotypic (identical monosaccharide units) sugar sequences.

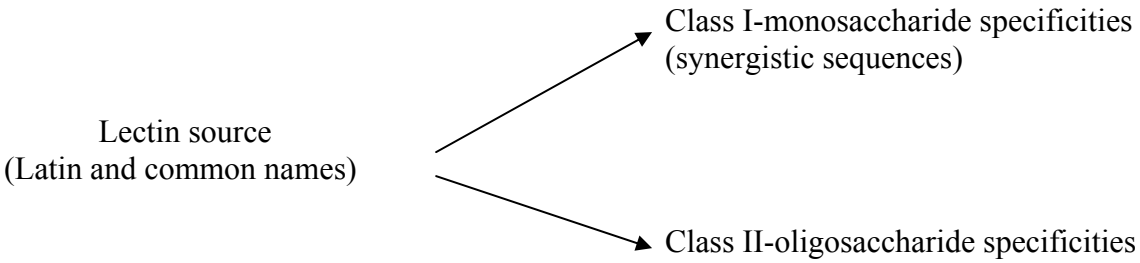
b) Class-II b (heterotypic endolectins):

These lectins have the highest affinity to heterotypic (different monosaccharide units) sugar sequences.

2.4.6 Nomenclature of lectins

A simplified method for the nomenclature of lectins was proposed by Gallagher (1984), depending on their carbohydrate-binding properties. In the proposed scheme, the lectin class and subclass are defined, followed by a description of carbohydrate-binding specificities. Latin names and common names of the source of the lectin should be stated together with accepted abbreviations in parenthesis.

The suggested nomenclature for each lectin is:



e.g. *Arachis hypogea*, peanut (PNA): Ia, D-Gal (Galβ1→3GalNAc).

According to their carbohydrate-binding specificity (Roth 1978, Goldstein and Poretz 1986) the lectins are classified into seven groups (table 2).

Table 2: Classification of lectins according to their carbohydrate-binding specificity (Goldstein and Poretz 1986).

Sugar specificity	**Source of lectin**	**Abbreviation**
D-mannose / D-glucose binding lectins	*Canavalia ensiformis* *Lens culinaris* *Pisum sativum*	Con A LCA PSA
N-acetyl-D-glucosamine-binding lectins	*Triticum vulgare* *Ulex europaeus* *Griffonia simplicifolia*	WGA UEA-II GSA-II
N-acetyl-D-galactosamine-binding lectins	*Dolichos biflorus* *Helix pomatia* *Glycine maximus* *Visea villosa*	DBA HPA SBA VVA
D-galactose-binding lectins	*Arachis hypogaea* *Griffonia simplicifolia* *Maclura pomifera* *Ricinus communis*	PNA GSA-I MPA RCA-I and RCA-II
L-fucose-binding lectins	*Lotus tetragonolobus* *Ulex europaeus*	LTA UEA-I
Sialic acid-binding lectins	*Limulus polyphemus*	LPA
Lectins with complex carbohydrate-binding sites	*Phaseolus vulgaris*	PHA-E and PHA-L

2.4.7 Endogenous Lectins

In addition to the plant lectins, many lectins of animal origin have been discovered. These lectins are either of invertebrate or of vertebrate origin. Noguchi (1903) found a lectin specific for N-acetyl-neuramine, in the hemolymph of the gastropod *Limulus polyphemus*. The first vertebrate lectin was found by Ashwell and Morell (1974), who discovered this

galactose-specific protein on the membrane of the liver cell of rabbit. During the last quarter of the last century, several endogenous lectins of vertebrate origin were found (Rüdiger, 1981; Barondes, 1986). Vertebrate lectins constitute a wide array of different sugar receptors (Gabius, 1987), which on the basis of their incorporation into membranes, can be categorized into integral membrane proteins and soluble intra- and/or extracellular tissue lectins. In the last few years, the significance of the vertebrate endogenous lectins became apparent. The interactions between the proteins and carbohydrates play indispensable roles in many biological processes (Frazier and Glaser, 1979).

As components of cell membrane, endogenous lectins play a great role in cellular interactions (Yolken et al., 1987). Therefore, they possess potential physiological significance in fertilization, development and in the pathogenesis of cancer (Gabius, 1987). Sinowatz and co-workers (1990) reported for the first time the histotopographical distribution of carbohydrate-binding proteins (endogenous lectins) in the bovine testis.

2.4.8 Applications of lectins

Blood typing and mitogenic stimulation of lymphocytes were the earliest and still widely used applications of lectins in the biological fields (Lis and Sharon, 1986). The labelled lectins are applied as reagents for the study of simple and complex carbohydrates in the solutions and on the cell surfaces. Lectins are used for the identification and separation of certain types of cell, for example, peanut agglutinin (PNA) interacts selectively with immature thymocytes. This represents the basis of the widely used method for the separation of these cells from the mature thymocytes by selective agglutination and affinity chromatography. Furthermore, the lectins have been used for the selection of lectin-resistant mutants of animal cells with altered glycosylation patterns. Lectins have been also widely used for mapping of central neuronal pathways (Lis and Sharon1986).

Lectins have been extensively used in histochemical and cytochemical studies (Peters and Goldstein 1979; Watanab et al., 1981; Lis and Sharon1986, Gabius et al., 1988). Lectin binding patterns exhibit changes in cases of embryonic differentiation, cell maturation, aging, metaplastic alterations, malignant transformation and other pathological conditions (Lis and Sharon, 1986). Experiments with lectins have also helped to localize the intracellular sites of protein glycosylation (Lis and Sharon1986).

2.4.9 Application of lectins for the characterization of the epididymis

Lectins have been used to demonstrate the distribution of glycoproteins in the epididymis in different species including humans (Arenas et al., 1996, 1998), hamster (Calvo et al., 1995, 1997), rats (Arya and Vanha-Perttula,1984, Ueda et al., 1998), mice (Lee and Damjanov, 1984, Bendahmane and Abou-Haila, 1997), boars (Zürcher, 1992), stallions (Kuhrau, 1993) and bulls (Arya and Vanha-Perttula, 1985a; Lindinger-Niederhofer, 1991; Rauscher, 1991).
Some differences were recorded in the lectin-staining pattern of various cell types of the epididymal epithelium of the adult Wistar rats. This variable reactivity indicating cellular specialization for the production of distinct glycoproteins (Arya and Vanha-Perttula 1984). Several lectins have a high specificity for certain cell types and could be used as histochemical probes for specific epididymal cells of mice, so that providing non traditional convenient means for light-microscopic identification of epididymal segments and cells (Lee and Damjanov1984).
A comparative study involved different species of rodents (gerbil, guinea pig, mouse and nutria) for lectin-staining pattern in the epididymis. The results revealed that the principal cells (PC) showed a lectin-stained Golgi region, apical surface, stereocilia and tubular contents (Arya and Vanha-Perttula, 1986). Lectins have been extensively used to localize the sugar residues of the sperm plasma membrane and to demonstrate changes occurring during their epididymal transport and after ejaculation (Sinowatz and Friess, 1983; Sinowatz et al., 1989). The extensive use of lectin histochemistry in studying the changes in the localization of sperm surface glycocomponents throughout the different portions of the male genital tracts, has led to a more precise characterization of the different parts of the ductus epididymis (Liu et al., 1991; Bains et al., 1993). The distribution of lectin staining in six regions of bovine epididymis showed some typical differences that were associated with the secretory and absorptive functions of the epididymis (Arya and Vanha-Perttula1985a; Rauscher, 1991).
Arya and Vanha-Perttula (1985a) have used the lectins for the demonstration of the glycoproteins in the bovine epididymis. The different epididymal segments showed remarkable variations in their lectin-binding pattern. Region I revealed a strong surface reactivity and a patchy reaction in PC. Regions II and III showed a strongly reactive apical Golgi zone and secretory material. Regions IV and V manifested a strongly positive sub-apically located Golgi zone with most lectins. Rauscher (1991) has also used the same types of lectins, which displayed a highly variable and a characteristic binding pattern in the epididymal epithelium. Lectin binding sites in the different segments of the epididymis

demonstrated clear-cut differences both in localization and in the intensity of reaction for individual lectins. PC of the proximal segments revealed a high degree of binding sites represented by the supranuclear Golgi zone, the apical surface and the stereocilia. Lectin binding sites of the corresponding cellular elements in PC of the distal epididymal segments could be visualized only with Con A and WGA. Furthermore, lectin-binding sites in the distal segments existed in the basal cytoplasmic region of PC and in the cytoplasm of the basal cells (BC) as well. Despite the use of the same types of lectins for demonstration of the glycoproteins with different sugar residues in the bovine epididymis, some differences in their binding pattern exist between the work of Arya and Vanha-Perttula (1985a) and that of Rauscher (1991).

2.5 Electron microscopic structure of the epididymis

2.5.1 General view

Both light and electron microscopic studies of the mammalian ductus epididymis revealed five types of epithelial cells: principal, basal, apical, and clear as well as the so-called halo cells (Piomboni, 1997). The principal cells in the initial segment of the epididymis in horses, cattle, pigs, sheep, dogs, cats and rabbits have an abundant, partly rough ER and a large Golgi complex. Small vacuoles with opaque content seem to be formed by the Golgi complex and move to the cell apex, where they empty their contents into the lumen by a merocrine mechanism (Nicander and Malmqvist, 1977). The epididymal duct epithelium lies on a distinct basal lamina. The latter is formed by three laminas: lamina lucida, lamina densa and lamina fibroreticularis. It represents the limiting structure between the epididymal epithelium and the periductal stroma (Schimming and Vicentini, 2001).

2.5.2 Species specific differences

The initial segment of the rat epididymis presents four distinct cell types: the PC, the BC, the so-called apical mitochondria-rich cells (AMRC) and halo cells. PC are tall columnar extend the full thickness of the epithelium from basal lamina to the lumen. They have round or elliptical nuclei, very prominent Golgi apparatus and a free surface bearing long stereocilia. The latter are variable in diameter and have a tendency to branch. (Hoffer et al., 1973). The plasma membrane between the bases of the stereocilia is highly irregular in contour and has numerous shallow depressions and deeper pit-like invaginations. The plasma membrane covering the stereocilia is somewhat thicker than that of the lateral and the basal cell surfaces

and much thicker than the membranes of the Golgi complex and endoplasmic reticulum (ER). Typical coated vesicles of various sizes are frequently seen in the apical cytoplasm and to a lesser extent in the supranuclear region. These pinocytotic vesicles remain distinguishable from surrounding vesicular elements of the ER by their thicker membrane and by the absence of a content of appreciable density. The existence of multivesicular bodies (MVBs) is one of the most characteristic features of the PC of the epididymis. Membrane-bound bodies containing a homogenous dense matrix exist in limited number. They represent primary lysosomes. ER is extensively developed with a consistent pattern of regional distribution. Parallel cisternae of the typical rough type are concentrated in the basal and paranuclear regions, whereas the apical cytoplasm is overcrowded with tubular elements possessing large calibre and covered by widely separated ribosomes. The PC have an elaborate Golgi apparatus, which consists of several stacks of cisternae. The outermost cisternae of the forming face are fenestrated and therefore appear discontinuous in section. They present a regular pattern of circular pores or fenestrae when viewed en face. The periphery of the deeper lying cisternae is also fenestrated. The lumen of most of the Golgi saccules appears empty. Both smooth surfaced and coated vesicles are numerous in the vicinity of the Golgi apparatus, but there are no condensing vacuoles or other evidence of concentration of a secretory product within the Golgi element or its associated vesicles.

The mitochondria of PC are very long and occasionally branched. They have numerous lamellar cristae, which are generally oriented perpendiculars to the long axis of the organelle. The mitochondrial matrix is of moderate density and occasional matrix granules observed. Mitochondria may be found anywhere in the cytoplasm but frequently concentrate along the lateral cell membranes, where they are generally oriented parallel to the longitudinal axis of the cells (Hoffer et al., 1973). Peroxisomes were immunolocalized in both PC and BC of the rat epididymis, frequently showing irregular profiles and a diameter of about 100 nm (Reisse et al., 2001). They may be engaged in ether lipid biosynthesis and consequently in the production of plasmalogens. The latter are a main component of spermatozoon membrane, playing a crucial role in sperm maturation (Reisse et al., 2001).

Halo cells have blunt processes, which extend between the PC, but have no complex interdigitation or other specialized attachment with epithelial cells. The nucleus is often indented and contains abundant heterochromatin. Elements of rER are infrequent, large mitochondria and occasional MVBs are also present. Free particles of ribonucleic proteins and occasional polyribosome rosettes are generally dispersed throughout the cytoplasm (Hoffer et al., 1973). PC of the initial segment of rat epididymis present two types of ER: the

heavily granulated, flattened type seen in the infranuclear and juxtanuclear regions, and the distended tubular, sparsely granulated type, showing only few ribosomes, seen interlaced with the Golgi complex in the supranuclear region and at the apical pole of the cell (Hermo et al., 1991). The sparsely granulated ER approximates the Golgi stack on its cis- and trans-faces. On the cis-face of the Golgi stack, the sparsely granulated ER cisternae show the usual finger- or bud-like protrusions directed toward the cis element of the Golgi stack and around which numerous small vesicles or membranous tubules are clustered. On the trans-aspect of the Golgi stacks, several trans-Golgi networks are observed often in close apposition to the sparsely granulated ER cisternae. The trans-Golgi networks are variable in appearance but often show sporadic electron-lucent dilations along their length that are comparable to uncoated vesicles of similar size and appearance found in the trans-region of the Golgi apparatus and at the apical pole of the cells. Such vesicles appear to arise from the trans-Golgi networks that eventually undergo fragmentation, giving rise to secretory vesicles (Hermo et al., 1991).

The intermediate zone, situated at the proximal end of the rat epididymis, is a wedge-shape area lodged peripherally between the initial segment and the proximal part of the caput epididymidis. The most distinctive feature of many PC of this zone is the presence of apically located vacuoles referred to as giant endosomes due to their large size and because they readily incorporated tracers introduced into the lumen of the epididymal duct and were acid phosphatase negative. Giant endosomes contain electron-dense granular patches. They appear to be formed by the progressive fusion of small, medium and large endosomes. In the supranuclear region, MVBs and lysosomes are present. Although smaller than the giant endosomes, MVBs and lysosomes contain electron-dense patches. It is suggested that giant endosomes fragment into smaller units corresponding to MVBs, which gradually transform into lysosomes. The presence of such large giant endosomes suggests that the early events in endocytosis are slower processes in the PC of this zone compared to other regions (Hermo, 1995).

The epididymal duct of the mouse is divided into five segments. Segments I, II and III constitute the head, segment IV constitutes the body and segment V constitutes the tail (Abe et al., 1983). PC decrease in height distalwards from the head to the tail. They also vary in ultrastructural details in the different segments. The microvilli are about 20 μm in length in segment I and they decrease distally to approximately 3 μm in segment V. A well-developed Golgi apparatus is seen in the supranuclear cytoplasm. Mitochondria are generally elongated in shape and scattered throughout the cytoplasm. ER in the head region is generally vesicular

and is distributed throughout the cytoplasm. The vesicular ER is rarely studded with ribosomes. The latter increase in number distalwards from segments I to III. In segments IV and V, the cisternae of rER exhibit flattened profile and are seen in the basal cytoplasm, around the nucleus and along the lateral surfaces of the cell. A large number of rER is arranged in lamellar forms, particularly in the infranuclear cytoplasm. MVBs were seen in PC of all segments of the epididymal duct. They are usually located in the supranuclear cytoplasm and are especially frequent in segments II and IV. The MVBs are approximately 0.5 μm in diameter in all segments except segment II where they are twice as large as in the other segments. MVBs contain varying numbers of vesicles and a matrix of variable density. Dense bodies are seen in the supranuclear cytoplasm in segment I and in the infranuclear cytoplasm in segments III, IV, and V. Few dense bodies were found in segment II. The dense bodies in PC show characteristic figures for each segment. The apical cytoplasm just under the luminal surface lacks the ER and mitochondria, but contains round coated vesicles. The coated vesicles are of two types, small and large. In segments I, II and III, the PC contain both small and large vesicles, however, the large vesicles are more numerous. In segments IV and V most of the coated vesicles are of the small type. The luminal surface occasionally has coated invaginations or opened coated vesicles between the adjacent stereocilia. The invaginations have almost the same diameters as the large coated vesicles in the first three segments and the small coated vesicles in the other two segments. Non-coated round vesicles, similar in size to the coated vesicles, are also seen in the apical cytoplasm and are three-times or more numerous as the coated vesicles (Abe et al., 1983).

The epithelium of the hamster epididymis is composed of several cell types. The PC are the most numerous and are accompanied by the small BC with few organelles and by halo cells. The proximal part of the epididymis contains AMRC. In the more distal parts of the epididymis, clear cells form a part of the epithelium (Flickinger et al., 1978). The epithelium of the initial segment is very tall and surrounds a small lumen containing few sperm. The apical surface of the PC is provided by long microvilli and presents pits and short caveolae. Abundance of vesicles and vacuoles in the apical cytoplasm is the most characteristic feature of the cells of the initial segment. Numerous microtubules and tubular profiles of sER are interspersed among the vesicles. A well-developed Golgi apparatus in the supranuclear cytoplasm has been seen associated with many vacuoles with a low-density content. Irregular anastomosing profiles of rER occupy the basal cytoplasm and extend into the perinuclear region. The lumina of these tubules are distended with a fine flocculent material

of moderate density; however some cells of this segment present dense spherical condensations of material in the lumen of the basally located rER. Lysosome-like structures and MVBs are concentrated in the supranuclear cytoplasm, whereas mitochondria with transversely oriented cristae are distributed throughout the cytoplasm. The nuclei are roughly circular and contain much euchromatin. Junctional complexes are present between the cells next to the lumen (Flickinger et al., 1978). The BC of the initial segment of hamster epididymis are oval to pyramidal shaped with heterochromatic nuclei and scanty cytoplasmic organelles. Halo cells are also seen and present a similar morphology to those of other species (Flickinger et al., 1978).

The caput epididymis of hamster is lined by a tall columnar epithelium and the lumen contains many more sperm than the initial segment. Small vesicles of both the smooth and coated type are found in the apical cytoplasm but occupy only a narrow zone under the surface. The presence of large clear apical vacuoles, MVBs and apical vesicles with predominantly electron-lucent content is an outstanding characteristic of PC of the caput region. However, some vacuoles and MVBs exist deeper in the cytoplasm with a denser content of flocculent texture. The Golgi complex of PC is very well developed. A myriad of vesicles and small vacuoles are associated with the large stacks of saccules. Numerous cisternae of rER occupy the basal cytoplasm, but in contrast with the initial segment, the profiles are narrow and contain little intracisternal material. sER and some scattered elements of rER are seen apical to the nucleus (Flickinger et al., 1978).

A variety of large, oval, lightly-staining cells with an electron-lucent cytoplasm and a pale nucleus exist in the epithelium of caput and corpus of hamster epididymis. They are usually in contact with the basal lamina but do not reach the lumen. These cells contain long, widely separated cisternae of rER but lack other distinguishing characteristics. They may represent light cells that are inactive or less developed than those of the cauda epididymidis (Flickinger et al., 1978).

The PC of the corpus region of hamster epididymis differ from those of the caput mainly in that the large apically located vacuoles are less frequent. The height of the epithelium of the cauda epididymidis is decreased with a further decline in the abundance of apical vesicles and vacuoles. The apical vesicles are scattered sparsely through a very narrow zone of cytoplasm. The Golgi apparatus occupies a large part of the supranuclear cytoplasm and dense lysosome-like structures often exist immediately apical to the nucleus. The ER consists of narrow cisternae with little intracisternal content. They exist mainly in the basal and perinuclear parts of the cells. However, their number is restricted in comparison to that

of proximal epididymal segments. Tubular and vesicular profiles of sER are most frequently encountered in the apical parts of the cells (Flickinger et al., 1978). Large light cells are abundant in the epithelium of the cauda epididymidis and are especially numerous in distal portions. Their surface contains few microvilli but exhibits long sinuous processes. Small vesicles, tubules and invaginations of the cell surface, all of which have clear interiors, occupy the apical end of the light cells almost entirely. Large numbers of membrane-bound, moderately dense bodies that resemble lysosomes are found more basally, in the supranuclear and perinuclear areas. These lysosome-like bodies of the light cells attain a particularly large size in the distal cauda, ranging up to 10 μm in diameter (Flickinger et al., 1978).

The fine structure of the rabbit epididymal duct shows regional differences in the PC characteristics. The cells of the initial segment reveal selective micropinocytosis with transfer of surface membrane into large MVBs, whereas those of the middle segment show profuse pinocytosis of opaque material in the lumen and of the pinocytotic markers and abundant lysosomes. The terminal segment shows some micropinocytosis only with small MVBs. There is some evidence of eccrine protein secretion in the initial segment, but most of the luminal material is taken up again in the proximal part of the middle segment. (Nicander and Plöen, 1979).

The PC of segment II of the epididymal duct of guinea pig exhibit numerous large lipid droplets and abundant agranular ER, which is frequently arranged in concentric whorls around one or more of the droplets. The quantitative biochemical studies revealed that the segment II contains more cholesterol and cholesterol ester than the segment I and III. Taken together, the ultrastructural and biochemical characteristics suggest that the segment II exhibits steroidogenic activity (Hoffer and Karnovsky, 1981).

The epithelial lining of the feline epididymal duct consists predominantly of columnar PC, BC dispersed among them and scattered AMRC. The latter are observed only in the proximal part of the duct. Migratory lymphocytes are frequently seen at different levels of the epithelium. Typical junctional complexes adjoin epithelial cells at their luminal endings. More deeply, typical desmosomes and membrane interdigitations are also seen connecting adjacent cells (Arrighi et al., 1986). The apical region of the PC of the cat epididymis presents long stereocilia, caveolae, vesicles, MVBs, sparsely granulated profiles of ER, free

ribosomes and sparse elongated mitochondria. Groups of small membrane-bound vesicles of variable diameter exist in the luminal space (Arrighi et al., 1986). The PC of the cat epididymis present a large Golgi apparatus occupying a wide zone in the supranuclear cytoplasm. Euchromatic nuclei often showing irregular profiles and sometimes nucleoli are prominent. The ER lies mainly in the perinuclear and infranuclear zones of the cytoplasm. ER exhibits variable appearance according to its localization: in the apical region, it is composed of a lot of cisternae and vesicles, which are only sparsely granular, while at the basal and the perinuclear compartment it consists of flattened parallel cisternae. The content of the ER, when evident, is moderately electron-dense flocculent. In the nuclear neighbourhood and always in close vicinity to ER profiles, an accumulation of electron-dense particles can be seen. Their appearance is variable somewhere resembling glycogen β particles, somewhere ribosomes. Cytoplasmic granules are evident throughout the cell. They are heterogeneous in size, form and osmiophility, due to their probable lysosomal nature. Typical residual bodies can frequently be seen (Arrighi et al., 1986). Mitochondria of the PC of the feline epididymis are heterogeneous and distributed throughout the cytoplasm. In addition to typical forms, highly unusual mitochondria have been encountered. The unusual forms contain crystalloid inclusions consisted of groups of parallel wavy lamellae, alternating with clear spaces. Sometimes, only the presence of cristae parallel or transversal to the crystalloid inclusion and the double limiting membrane permit recognition of the mitochondrion. Sometimes, dense round granules without a limiting membrane are detectable among the lamellar stacks (Arrighi et al., 1986).
The BC of the feline epididymis are rounded, often irregular in shape due to cytoplasmic protrusions extending between the adjacent PC. Their cytoplasm contains a clear matrix and scarce organelles such as mitochondria, free ribosomes, some profiles of rER and variably oriented filament bundles. Sometimes, residual bodies can be seen. The AMRC are very rare possessing an expanded funnel-like adluminal part. Their apical border presents short microvilli and the cytoplasm contains numerous mitochondria. Membrane-bound and sometimes irregularly shaped granules can be seen (Arrighi et al., 1986).

The cellular population of the canine epididymal epithelium is formed mainly by principal, basal, apical and clear cells (Schimming and Vicentini, 2001). The PC are cylindrical in shape and extend the distance from the basal lamina to the ductular lumen. They represent the predominant cell type in the epididymal epithelium (Chandler et al., 1981). PC have elongated to oval nuclei and stereocilia on the apical surface. The cytoplasm of these cells

contains many endosomes, vesicles and MVBs in the apical cytoplasm (Schimming and Vicentini, 2001). A well-developed Golgi apparatus is found in the supranuclear area of the cytoplasm (Chandler et al., 1981). The ER is also well-developed and concentrates in the perinuclear area. Many mitochondria can be seen among the cisternae of rER as well as along the lamellae of the Golgi complex (Schimming and Vicentini, 2001). Junctional complexes are well formed between neighbouring plasma membranes in the apical parts of the PC. Furthermore, the cell membranes appear highly interdigitated (Chandler et al., 1981). The AMRC exist in all three segments of the canine epididymis. They have an elongated nucleus located close to the luminal border of the epithelium. They show a luminal border sparsely provided with microvilli. Their cytoplasm contains a fair number of mitochondria with cristae (Schimming and Vicentini, 2001). The BC are usually small, rounded or elongated, with an irregular outline. They are basally located and never reach the luminal surface. They possess a large irregular outlined nucleus and scanty cytoplasm with scarce organelles. Their cytoplasm presents a poorly developed Golgi apparatus, some mitochondria and dispersed cisternae of ER (Schimming and Vicentini, 2001). Their cytoplasm contains accumulations of glycogen and bundles of filaments. Micropinocytosis is observed at the basal plasma membrane (Chandler et al., 1981). The clear cells are only seen in the middle and terminal segments. Their name is indicative for their morphology, where they present an apical region with numerous poorly dense vesicles of various sizes and a large highly vesiculated supranuclear region with some electron-dense bodies and lysosomes. The halo cells were not seen in the canine epididymis (Schimming and Vicentini, 2001).

The epithelial cell population of the equine epididymis is formed mainly by the PC, BC and occasionally intraepithelial leukocytes (López et al., 1989). In addition to the aforementioned three types, AMRC represent a fourth cell type (Arrighi et al., 1993). The PC are found along the whole length of the epididymis, showing some differences in their morphological and cytochemical characteristics in various regions, such as the occurrence, distribution and content of the vesicles, vacuoles, electron-dense structures and organelles (López et al., 1989). The PC of the caput of the equine epididymis have long apical microvilli with caveolae and micropinocytotic vesicles between them (Arrighi et al., 1993). The supra-nuclear cytoplasm presents mitochondria, polyribosomes, sparsely granulated ER and an abundant Golgi complex. The lumen of the ER is distended and presents a fine flocculent material (López et al., 1989). The basal portions of the cells are characteristically crowded

with mitochondria and in small numbers, by osmiophilic membrane-bound round or oval granules. Lysosomes and dense bodies are sometimes prominent throughout the cytoplasm. The nucleus is basally situated and presented one or two nucleoli (Arrighi et al., 1993).

A singular finding in the caput region is the presence of intraluminal phagocytic cells containing a significant number of spermatozoa fragments in their cytoplasm. These spermatozoa are apparently undergoing a process of degeneration (López et al., 1989).

The outstanding characteristic of PC of equine corpus epididymidis was the presence of clear apical vesicles and vacuoles with different sizes and texture of their content. MVBs of different sizes are also seen singly or in clusters in the apical region of these cells. The majority of these bodies are large, with a flocculent, low density, vesicular or electron-dense matrix. The apical cytoplasm of these cells appears characteristically vacuolated and presents some mitochondria, ribosomes and polyribosomes. In the distal corpus region the number of dense granules and vacuoles diminishes in the apical cytoplasm of the PC. The Golgi complex and the ER are more developed. In the perinuclear and infranuclear regions, small granules of different sizes and densities are observed (López et al., 1989). The region of the cauda epididymidis is characterized by a decrease of epithelial height concomitantly with a remarkable increase of the luminal diameter. The epithelium is more cuboidal and formed by PC and BC. There is a further decline in the number of apical micropinocytotic vesicles, vacuoles and microbodies of the PC. In contrast to the proximal segment of the epididymis, ER of the PC consists of narrow cisternae with little intracisternal content. A prominent Golgi complex occupies a large part of the supranuclear cytoplasm (López et al., 1989). Intranuclear inclusions, which may be crystalloid, multilamellar and needle-like or in the form of groups of globules surrounded by a single membrane are frequently seen. Sometimes both forms exist together (López et al., 1989; Arrighi et al., 1993). The BC of the equine epididymal epithelium are round and poorly differentiated. Their nuclei are euchromatic and never contain inclusions. The cytoplasm is rather poor in organelles, but does occasionally contain bundles of osmiophilic filaments. Frequently a cilium emerges from one centriole and insinuates in the extracellular space. Residual bodies are often present and sometimes occupy large areas of the cytoplasm. In some cases residual matter is so abundant that it completely masks the cytoplasm. The AMRC are slender funnel-shaped and distributed in small numbers along the entire epithelium. They reach the lumen with an expanded cytoplasmic area where mitochondria are abundant (Arrighi et al., 1993). Intraepithelial cavities are lined by cells whose cytological features are clearly similar to those of the PC, although the organelles are reduced. These cells have short microvilli,

which project into the lumina of the cavities. Granulated lymphocytes and sporadic macrophages are detectable along the entire epithelium (Arrighi et al., 1993).

The ultrastructure of many PC in the cauda epididymidis of water-buffalos (Santos and Dolder, 1999) reveals, in many cells, long, curved paracrystalline structures that are quite large and frequently encountered in the cytoplasm, usually near the nucleus. Concomitantly or not, smaller rod-like, hexagonal or curled structures can be found intranuclearly. Both cytoplasmic and intranuclear crystalloids are made up of a sheath of parallel filaments. These paracrystals may appear as thin, regularly spaced filaments that are associated with fine, evenly spaced subunits. Occasionally, the association of paracrystalline structures with membranes similar to the ER are observed, but no membranes are consistently found in close contact with the nuclear crystalloids. It was postulated that both structures were proteinaceous and may represent stored enzymes or substances present in the intraluminal fluid, which are absorbed and initially stored in numerous intraepithelial vacuoles of the corpus and cauda epididymidis of the buffalo-bull (Santos and Dolder, 1999).

The PC throughout the epididymis of monkey (*Macaca mulatta*, *Macaca fasicularis*, and *Macaca arctoides)* exhibited the morphological features of a cell type that is actively involved in absorption and secretion (Ramos and Dym, 1977a). The apical surfaces of PC are covered with long stereocilia, which often branched near their base. The core of each stereocilium contains bundles of closely packed fine filaments, which extend several micrometers into the cytoplasm. A consistent and conspicuous feature in the lumen of the monkey epididymal duct is the presence of large numbers of membrane-bounded vacuoles or spheres of varying sizes. These structures are usually observed in the distal head and body regions but not in the initial segment or the tail. Frequently many small spheres are found between the stereocilia, adjacent to the apical plasmalemma or in deep invaginations of the latter. These invaginations are called caveolae (Nabeyama and Leblond, 1974). These invaginated regions of the plasmalemma are coated with a fuzzy material that may be a stage in the formation or breakdown of the numerous micropinocytotic coated vesicles present in the apical cytoplasm. It is not uncommon to find direct continuity between the coated vesicles and the caveolae of the apical cell membrane (Ramos and Dym, 1977a). The rER of the PC of the monkey epididymis is particularly well-developed. Some of the vesicular and tubular profiles are scarcely covered with ribosomes, thus some parts remain smooth. Membranes of rER often surround mitochondria and lipid droplets. Mitochondria are of

different sizes with typical lamellar cristae. They tend to aggregate in clusters in the basal zone of the cytoplasm. A well-developed Golgi apparatus is usually observed in the supranuclear cytoplasm but does not appear to be as extensive as in other species (Ramos and Dym, 1977a). Lysosomes tend to accumulate in the supranuclear zone but occasionally in the paranuclear and basal region of the cytoplasm. The supranuclear cytoplasm of the PC is overcrowded with a variety of cellular organelles and inclusions; their occurrence and distribution varies along the epididymal duct. MVBs are present in apical cytoplasm but they are more numerous in the supranuclear Golgi region. Furthermore, lysosomes, membrane-bounded granules of various densities, free ribosomes, polyribosomes and mitochondria are also seen (Ramos and Dym, 1977a). The infranuclear cytoplasm is normally filled with large clusters of mitochondria and numerous small, membrane-bounded dense granules of different sizes and densities. These cellular inclusions are found in close proximity with large clusters of small mitochondria and to the subepithelial and peritubular capillaries. The intriguing feature of these dense granules is their resemblance to secretory granules of endocrine glands (Ramos, 1980). This characteristic feature of the infranuclear region of the PC is similar throughout the whole length of the epididymal duct and appears to be unique to the macaque monkey (Ramos and Dym, 1977a). The nuclei of the PC become progressively more infolded in the regions of distal head, body and particularly of the tail of the monkey epididymal duct. The extreme lobation of the nucleus of the PC in the tail region is a very characteristic feature of this segment. There is a high proportion of euchromatin in the nucleoplasm and prominent nucleoli. Low electron-dense prominent spherical intranuclear inclusions are observed, which consist of very fine filaments frequently arranged concentrically around a dense core (Ramos and Dym, 1977a). Adjoining PC of the monkey epididymis are connected at their luminal surfaces by occluding junctional complexes. The lateral cell membranes are relatively straight in the apical two-thirds of the cell but become highly irregular and interdigitated towards the basal lamina. Fine filaments are also apparent in the apical cytoplasm in the vicinity of the junctional complexes but they do not extend across the cytoplasm of the apex to form a terminal web. Desmosomes and associated filaments are observed along the lateral cell membranes, more frequently in the upper two-thirds of the cells (Ramos and Dym, 1977a). The AMRC are found consistently in the epididymal epithelium of the macaque monkey (Ramos and Dym, 1977a). They are tall electron-lucent columnar cells with cytoplasm that extends from the basal lamina to the luminal border. Each cell is quite narrow throughout most of its length but it appears to widen near the lumen. Their luminal surface contains only a few short microvilli. The

nucleus located within the apical third of the cytoplasm, is usually round or oval and exhibits shallow infoldings of its envelope. Unlike those of the PC, the nuclei do not vary in shape and degree of lobation in the different regions. Each nucleus contains numerous dense chromatin clumps and a prominent nucleolus. The outstanding feature of the AMRC is the existence of large number of mitochondria concentrated in the apical cytoplasm and around the nucleus. The mitochondria are of various shapes and sizes. The Golgi apparatus consists only of a few stacks of cisternae and saccules with a correspondingly small number of associated vacuoles and vesicular elements. Rough and smooth ER are very scanty, whereas free ribosomes, lysosomes, some lipids and other inclusions are also found in small amounts (Ramos and Dym, 1977a). Small BC resting on the basal lamina form a layer throughout the length of the monkey epididymal duct (Ramos and Dym, 1977a). Their cytoplasm does not extend to the luminal surface. The nucleus is round or ovoid, with or without deep invaginations with a little heterochromatin and few nucleolar elements. Intranuclear vacuoles are not uncommon especially in the tail region. Frequently, BC are seen to contain autophagic vacuoles, pigment granules and numerous electron-dense bodies. Profiles of poorly developed rough and smooth ER, a small Golgi apparatus and a few mitochondria are common cytological features of these cells. One noteworthy feature of them are the complex interdigitations of the plasma membrane with the adjacent PC. Desmosomes are usually found in these interdigitations and, in addition, pinocytotic infoldings in the lateral and more frequently at the basal plasmalemma are observed, suggesting uptake or release of material (Ramos and Dym, 1977a). Halo cells or IEL are morphologically similar to circulating lymphocytes and IEL in other organs. These cells are found at all levels in the monkey epididymal epithelium throughout the length of the duct (Ramos and Dym, 1977a). Some of them are small whereas others are large containing more cytoplasm than the typical ones. The cells usually possess a round or slightly indented nucleus with abundant heterochromatin and a thin rim of pale cytoplasm. The nuclei are occasionally highly infolded. The cytoplasm contains a few mitochondria, small amounts of smooth and rough ER and some dense granules. A frequent feature of this cell type is cytoplasmic pseudopodia that extend between PC (Ramos and Dym, 1977a).

The PC are the most numerous cell types lining the human ductus epididymis. Their height varies along the duct (Piomboni, 1997). They are characterized by a well-developed Golgi apparatus in the supranuclear zone of the cytoplasm and by an abundant network of ER in the infranuclear and basal cytoplasm. Their apical cytoplasm harbours numerous

micropinocytotic vesicles, MVBs, lysosomes, coated vesicles, lipid droplets and mitochondria. In the region of the caput, the nucleus exhibits an elongated form with the longitudinal axis parallel to the main cell axis. From distal part of the caput and distalwards, invaginations of the nuclear membrane become evident, particularly in the region of the cauda epididymidis. The BC are small round cells dispersed among the PC resting on the basal lamina of the epididymal epithelium. They contain few organelles and are slightly electron-dense. It was proposed that they may be stem cells (Piomboni, 1997).

2.5.3 Electron microscopic structure of the bovine epididymis

2.5.3.1 Efferent ductules

Wrobel (1972) mentioned that the epithelium of the bovine efferent ductules is simple columnar with scattered basal cells. Columnar cells include two categories, namely: ciliated and principal (nonciliated) cells. In the apical half of the epithelium, lateral cell membranes of neighbouring ciliated and principal cells are closely aligned together, however, in basal half of epithelium cells are often separated by wide intercellular spaces. Characteristic junctional complex and relative many desmosomes are found to bind the neighbouring cells together in the apical half of the epithelium, however, desmosomes are found only rarely in the basal region of the epithelium. Basal cells possess relatively electron-dense cytoplasm with free ribosomes, inconspicuous Golgi apparatus, ill-developed endoplasmic reticulum and few small mitochondria. They adapt themselves between the basal membrane and basal portions of principal and ciliated cells through expressing short stumpy cytoplasmic processes. Direct contact between basal cells and basal membrane is encountered only in little extent due to insertion of distal extensions of principal cells between them and the basal membrane (Wrobel, 1972).

All principal cells possess common ultrastructural characteristics. The nucleus is less electron-dense than those of ciliated and basal cells. An ill-developed Golgi apparatus is found juxtanuclear, endoplasmic reticulum is less developed and long mitochondria are found next to lateral cell membrane and in infranuclear area. Principal cells exist in two functional stages. The apices of the first group are equipped with large number of straight slender microvilli. Apical area of cytoplasm present a well-developed endocytotic apparatus represented by canaliculi, pinocytotic vesicles, coated vesicles as well as small electron-lucent smooth vesicles. Fusion of smooth and coated vesicles is often encountered with the result of formation of a large vacuole in supranuclear cytoplasm, the content of a vacuole is

predominantly electron-lucent, and however, flocculent granular material and smooth membranous fragments are found. Near from the nuclear region, vacuolar size is decreasing while the content of the vacuoles condensed. Principal cells of the second group are characterized by occurrence of apical protrusions. In these cells, vacuoles are rarely found in supranuclear cytoplasm; however, dense osmiophilic granules are abundant in the whole cytoplasm. In contrast to the remainder cell body, apical protrusions possess lighter cytoplasmic matrix, which is free from organelles, but not from electron-dense globules. The surface of the protrusions is generally free from microvilli. The endocytotic apparatus is less developed and formed only from few short canaliculi which exist between some stumpy microvilli directly basal to the site of the protrusion. However, Goyal and Hrudka (1980; 1981) recorded that the epithelium of bull efferent ductules consists of ciliated and nonciliated cells. Based upon the cytological characteristics, nonciliated cells are classified into three subtypes: type I cells (contain neither granules nor vacuoles), type II cells (abundant in granules) and type III cells (plenty of vacuoles). The luminal surfaces of nonciliated cells possess either a uniform microvillous border or an alternation of microvillous border and apical protrusions of various sizes (Goyal and Hrudka, 1980). These protrusions may contain granules or vacuoles and may be found released in the lumen along with granules and vacuoles (Goyal and Hrudka, 1981). Nonciliated cells possess an endocytotic apparatus beneath their microvillous border. This apparatus is composed of a variable number of microcanaliculi, micropinocytotic vesicles, coated vesicles and vacuoles. The nuclei of nonciliated cells are euchromatic and located in the basal third of the cells (Goyal and Hrudka, 1980; 1981). Nonciliated type II cells are characterized by existence of round, mostly homogenous and membrane-bound granules, which seem to fill the cytoplasm. The size of these granules increases from basal to luminal end of the cell. Variation in their shape, number, size and density are not uncommon among cells or even within the same cell. Granules originate from expanded rER. Thus the close relation between these granules and both rER and mitochondria is evident (Goyal and Hrudka, 1981). Nonciliated type III cells are characterized by occurrence of vacuoles in their supranuclear area. These vacuoles show small flocculent and membranous content indicating low density phospholipids. They possess a characteristic pattern of arrangement as stacks in a peg-and-socket manner. Mitochondria present typical transverse cristae are found as a row along the lateral cell membrane. Sporadic profiles of rough endoplasmic reticulum are encountered, whereas Golgi cisternae enclose pale contents similar to that of the vacuoles (Goyal and Hrudka, 1981).

Ciliated cells are distinguished not only by the cilia but also by the apical position of their nuclei, which are ovoid and heterochromatic (Goyal and Hrudka, 1981). Ciliated cells present many mitochondria, small Golgi apparatus and a few irregularly-shaped dense bodies in their supranuclear cytoplasm. Endoplasmic reticulum is inconspicuous. Their apical surfaces are equipped with typical kinocilia and always a few slender microvilli are scattered in-between (Wrobel, 1972). Elements of the endocytotic apparatus are scarce. Mitochondria are abundant both in apical (they are presumably provide the ciliary movement with energy) and basal regions and contrary to those of nonciliated cells they possess fewer and mostly longitudinally oriented cristae (Goyal and Hrudka, 1981). Intraepithelial lymphocytes are found scattered throughout the epithelium and are characterized by a strongly heterochromatic nuclei and paucity of organelles. Intraepithelial macrophages are present with their pseudopodia insinuating between the epithelial cells and possess a high number of lysosomes and residual bodies (Goyal and Hrudka, 1981).

2.5.3.2 Epididymal duct

The epithelium of the bovine epididymis consists of principal and basal cells as well as IEL and IEM. In addition to the aforementioned cell types, AMRC are found in the segments II-IV (Sinowatz, 1981; Goyal, 1985).

2.5.3.2.1 Segment I

The high columnar PC represent the majority of the cellular element of the epididymal epithelium (Goyal, 1985). The height of PC in this segment varies from 65 to 120 µm (Sinowatz, 1981). Their stereocilia are about 17 µm long and 1 µm thick and may be branched (Sinowatz, 1981; Goyal, 1985). Furthermore, cytoplasmic protrusions exist on the luminal surface and contain short strands of smooth endoplasmic reticulum (sER) and few ribosomes (Sinowatz, 1981). The apical cytoplasm, between the stereocilia, presents many pinocytotic invaginations; numerous small smooth-walled and coated vesicles, which may extend to occupy approximately the half of the supranuclear zone of the cytoplasm (Goyal, 1985). The content of these vesicles is light or moderate electron dense and on sporadic occasions, this material appears as condensed electron dense granules. Among these small vesicles exist larger vacuoles as well as numerous short profiles of ER, which are predominantly of the smooth type. However, the cisternae of ER are occasionally sparsely granulated. The length of cisternae and their content of ribosomes increase towards the nucleus (Sinowatz, 1981). Numerous microtubules occur between the vesicles and the ER.

They are arranged parallel to the long axis of the cell and are evidently in continuity with the stereocilia (Sinowatz, 1981; Goyal, 1985). Large numbers of small MVBs are seen in the apical and supranuclear cytoplasm (Sinowatz, 1981). A complex Golgi apparatus dominates in the juxtanuclear zone. The centre of this array incorporates an apparently homogenous zone of numerous small vesicles, which bud from the inner cisternae as short smooth-walled cisternae with moderately electron dense content. Longer cisternae are peripherally and concentrically arranged. Golgi cisternae are connected by cross-links, especially in the peripheral areas (Sinowatz, 1981). Peripheral to the Golgi complex, a large number of rER cisternae containing moderate electron-dense material, exists especially closer to the lateral cell membrane (Goyal, 1985). Numerous mitochondria, a few cisternae of rER and many small vacuoles are seen in the infranuclear cytoplasm (Sinowatz, 1981).

The nucleus of the PC is oval in shape and occurs in the basal third of the cell body. It has a moderate content of heterochromatin, which is represented by a narrow rim at the periphery of the nucleus and as small islands throughout the karyoplasm. Many of the nuclei possess one or more compact nucleoli. The narrow cytoplasmic zone, which laterally embraces the nucleus (juxtanuclear zone), contains sporadic mitochondria with cristae as well as some cisternae of rER. The oval or elongated mitochondria predominate in the infranuclear zone. Basally, in addition to the mitochondria, various forms of numerous small vesicles and electron-dense granules are found (Sinowatz, 1981).

The basal cells (BC) have a size of 7 µm. They never reach the luminal surface. Their number reaches about one-fourth of the PC population. They sit on the basal lamina (BL) and their contact to it is restricted to a small area through their wedging among the narrow basal extensions (feet) of the PC. Their nuclei are kidney-shaped and are not infrequently deeply invaginated or even subdivided in two lobes. Their lightly stained cytoplasm contains, in addition to a small number of mitochondria with lamellar cristae, an inconspicuous Golgi apparatus, a few strands of rER and a large number of free ribosomes. The BC often show bundles of cytoplasmic filaments and have lipid droplets. Their contact with the neighbouring high prismatic PC is mediated by interdigitation of the cytoplasmic processes (Sinowatz, 1981).
Other cell types as macrophages and lymphocytes are occasionally encountered in the basal third of the epithelium. The pseudopodic cytoplasmic processes of macrophages are lodged between the high prismatic PC and the BC. Around their relatively small heterochromatic

nuclei, many mitochondria, a well-developed Golgi apparatus and rER are found. Peripheral to these organelles, exist dark globules, which probably represent phagosomes as well as various profiles of lysosomes. In the outermost cytoplasmic zone, smaller vesicles are found (Sinowatz, 1981).

The epithelium of the first segment as that of the other segments present small cells with dense round nuclei and a narrow peripheral cytoplasmic rim (Goyal, 1985). These are the intraepithelial lymphocytes (IEL). Different from macrophages, they occur at different levels of the epithelium; however they also exist in larger numbers in the basal area. They are often encountered in an immediate proximity with the subepithelial capillaries (Sinowatz, 1981). They are formerly described as "halo cells" (Reid and Cleland, 1957). Ultrastructurally they demonstrate many features of the lymphoid cells with very heterochromatic nuclei. The latter are round and have small and large indentations (Sinowatz, 1981). Their cytoplasm is clearly lighter than that of the neighbouring cells and contains fewer organelles (Goyal, 1985). The Golgi apparatus is small and consists of some flat cisternae and small vesicles as well. Furthermore sporadic cisternae of ER, large number of ribosomes and occasionally dense bodies and lipid droplets are found throughout the cytoplasm (Sinowatz, 1981).

2.5.3.2.2 Segment II

The height of the epithelium is uniform and its mean value reaches 70 µm. Stereocilia are arranged in a layer of 10 µm height. The apical cytoplasmic protrusions that exist in the first segment are absent (Sinowatz, 1981). In addition to the four cell types present in segment I, a small number of apical mitochondria-rich cells (AMRC) exists in the epithelium (Sinowatz, 1981, Goyal, 1985). The most characteristic feature of PC is the abundance of numerous vacuoles and MVBs especially in the apical cytoplasm (Goyal, 1985). In comparison to the first segment, the proportion of the larger vesicles has increased (Sinowatz, 1981). Numerous round granules of variable electron density are found between the Golgi zone and the nucleus as well as underneath the nucleus (Sinowatz, 1981). Both lipid droplets and the surrounding whorls of sER seen in segment I are absent (Goyal, 1985). Peripheral to Golgi zone, long cisternae of rER and some elongated mitochondria are located parallel to the longitudinal axis of the cell, especially closer to the lateral cell membrane (Goyal, 1985). Mitochondria are recognizable through their comparatively dense matrix and the transverse as well as the tubular cristae. In sporadic cases, branching of mitochondria could be observed (Sinowatz, 1981). Basally, there are small vesicles amid the mitochondria.

The infranuclear area appears different having a high content of sER (Sinowatz, 1981). The small BC are confined between the neighbouring borders of the PC. The contact of these BC to the BL is variable and depends on the extent of their contact area with the PC. Their organelles are inconspicuous as in the first segment. Lipid droplets are located immediately adjacent to the nuclear membrane. There is a clear topographic relation between lipid droplets and mitochondria (Sinowatz, 1981).

The AMRC exist in this segment and due to their round to oval nuclei they can be easily identified at the apical third of the epithelium, even at the light microscopic level (Sinowatz, 1981). Their light cytoplasm is well distinguishable from the denser PC i.e. there is a high contrast between the two cell types. The mitochondrion is the predominant organelle. It exists in a large number and is mainly found apical to the nucleus (Sinowatz, 1981; Goyal, 1985). Other organelles are only sparse, however a varying number of vacuoles in apical cytoplasm is frequently encountered (Goyal, 1985). The luminal surface of AMRC is provided with microvilli that are considerably shorter than stereocilia of the PC. The AMRC obviously do never reach the BL (Sinowatz, 1981). IEM appear in the infranuclear zone of the epithelium and are similar to that of the first segment. Also their number and appearance is corresponding to that of the first segment. IEL are similar to that encountered in the first segment (Sinowatz, 1981; Goyal, 1985).

2.5.3.2.3 Segment III

The mean epithelium height is 70 µm and stereocilia are 10 µm long. The apical surfaces confined in between the stereocilia of the PC present several micropinocytotic invaginations. The cytoplasm of the apical zone contains numerous vesicles and short strands of ER, which belong predominantly to the smooth type. Now and then sporadic profiles of ER carry ribosomes (Sinowatz, 1981). As in the previous two segments, numerous microtubules originate from the base of the stereocilia. In the juxtanuclear and in the infranuclear cytoplasmic zones, microtubules are also observed. These microtubules are oriented in bundles parallel to the longitudinal axis of the cell. Underneath the apical zone exist numerous strands of rER and some mitochondria (Sinowatz, 1981; Goyal, 1985). The structure of the mitochondria differs in various aspects from that of the proximal segments. In an electron-dense matrix, numerous less electron-dense crossly cut tubules are recognizable, however, the transverse cristae are found throughout the electron dense background (Sinowatz, 1981). The juxtanuclear oriented Golgi complex, which is

surrounded laterally by mitochondria and rER (Goyal, 1985) occupies a wide area above the nucleus (Sinowatz, 1981). Immediate to the Golgi apparatus, there are variable forms of granules mostly with a strong electron density. Similar granules are found underneath the nucleus (Sinowatz, 1981).

A characteristic feature of the segment III is the bizarre contour of the nucleus (Sinowatz, 1981). The nuclei are often subdivided in chromatin strands that are bound together throughout narrow bridges. Many of the nuclei have basket-shaped nucleoli. A few nuclei show fine filamentous material, which is longitudinally oriented, forming a compact structure (Sinowatz, 1981). As a result of the apical displacement of the nucleus, the infranuclear zone of the cytoplasm becomes wider than that of the previous segments (Sinowatz, 1981).

The BC essentially show a similar appearance and size as in the previous two segments, but the lipid droplets in the cytoplasm are markedly increased (Sinowatz, 1981). The number of IEM is obviously increased and they are found in the infranuclear and basal zones of the epithelium. However in sporadic cases, they are found in the apical zone of the epithelium (Sinowatz, 1981). The AMRC are also found in the third segment (Goyal, 1985). Their number may reach up to 10 cells per cross section of a tubule (Sinowatz, 1981). IEL exist in a similar number and appearance as in the previous two segments (Sinowatz, 1981; Goyal, 1985).

2.5.3.2.4 Segments IV and V

The lumen of the fourth epididymal segment is round or oval and contains many spermatozoa. The epithelial height reaches a mean of 80 µm. The fifth segment represents the distal corpus and differentiates from the fourth segment through its epithelial folding. The epithelial height reaches 70 µm at the base of an epithelial fold and 100 µm at its top (Sinowatz, 1981). The ultrastructural characteristics of the epithelium in both fourth and fifth segments are identical and consequently can be described together. The PC of both segments are provided with 10 µm long stereocilia, which arise from conical cell apices presenting smooth-walled and coated vesicles and occasionally large MVBs within the apical cytoplasm (Sinowatz, 1981).

Goyal (1985) described the segments II-IV together due to their histological similarities and recorded major differences in the PC between region V and the regions II-IV, including the absence of large cytoplasmic vacuoles and MVBs in region V. The size of the Golgi

complex is smaller in comparison to that of the epididymal head segments. The area immediately above the nucleus is occupied by rER (Sinowatz, 1981). Nuclei are either long expanded or oval in their form and contain often one or more honeycomb-like nucleoli. Noticeably is the existence of a few numbers of mitochondria in the supranuclear area; and a great number of them appear in the infranuclear and basal areas. The mitochondria are either elongated or not, infrequently, twisted. Their mitochondrial matrix has moderate electron density and show mainly transversal oriented cristae. Many of them contain additionally tubules. The lipid droplets are often in proximity or even in immediate contact with the mitochondria (Sinowatz, 1981).

The basal cytoplasmic area is particularly complex. The high prismatic PC with their cytoplasmic processes wedge between the BC. The contact between the latter and the BL is restricted to narrow areas (Sinowatz, 1981). The basal cytoplasm of PC contains bundles of filaments, mitochondria and a large number of smaller heteromorphic granules (Sinowatz, 1981; Goyal, 1985). The BC exist in larger numbers in the corpus epididymis than in the caput epididymis. The ratio between the BC and PC in the region of the epididymal body reaches 1: 3 (Sinowatz, 1981). It was not uncommon to find wide lateral intercellular spaces with short microvilli between a BC and the adjoining principal or basal cell (Goyal, 1985). Only very few AMRC can be observed in the region of the epididymal body (Sinowatz, 1981). However, when they are encountered they present a similar morphology as those of the previous segments (Goyal, 1985). Also, the numbers of the IEM are apparently reduced and the IEL are sporadically seen (Sinowatz, 1981; Goyal, 1985).

2.5.3.2.5 Segment VI

The sixth segment represents the epididymal tail. It is characterized by a wide lumen, which is densely crowded with spermatozoa. The latter are partially lost and washed away due to processing for the electron microscopy. The epithelium of the proximal one-third of this segment appears slightly folded. More distally the folds fade downward and the luminal contour becomes regular. The lumen is surrounded by an epithelium of approximately 50 µm heights (Sinowatz, 1981). In addition to the components of the endocytotic apparatus in the apical cytoplasm, dense granules and vacuoles with or without central minute granule are distributed throughout the cytoplasm of the PC. The vacuoles with central granules contain myelin-like material in the centre and are surrounded by many bell-shaped mitochondria (Goyal, 1985). Some autophagosomes are seen containing mitochondrial fragments (Goyal,

1985). The electron microscopic picture shows that the supranuclear cytoplasm possesses a characteristic stratification of the cellular organelles. The latter are somewhat different from that of the previously described segments. In the apical zone of the supranuclear area, the number of the vesicles that characterizes the previous segments decreases. This contributes certainly to the compact appearance of the apical zone of the sixth segment. Therefore, free ribosomes occur in a larger number, often in the form of polyribosomes. This is followed by a wide cytoplasmic zone characterized by many short rER strands (Sinowatz, 1981). The third zone of the supranuclear cytoplasmic area contains the Golgi complex. The latter is similarly developed as in segment V and consists of many stacks of cisternae as well as the peripherally oriented vesicles (Goyal, 1985). The nuclei of the PC demonstrate a similar profile to that of the third segment; they possess a very irregular contour. The nuclear materials are often subdivided into many longitudinally oriented chromatin fragments that are joined together through bridges. The nucleoli with their peripheral position are often found (Sinowatz, 1981). A unique feature of some nuclei of PC of this segment is the presence of filamentous inclusions that are approximately 1µm wide and extend up to three-fourths of the length of the nucleus (Goyal, 1985). Nearby the nucleus, electron dense lysosomal granules exist, which have variable sizes and different forms. Unlike to the proximal segments, a large number of mitochondria found around the nuclei. These mitochondria as well as those occurring in the infranuclear and basal areas, reveal a great variety of forms. In addition to the elongated and oval forms, there are also dumbbell-shaped and ring-shaped mitochondria (Sinowatz, 1981). Mitochondria with tubular cristae are abundant in supra- and infranuclear cytoplasm (Goyal, 1985). The BC are similar to that of the proximal segments in their size and forms (Goyal, 1985). However, their content of lipid droplets is considerably increased. A single cell can contain five droplets or more (Sinowatz, 1981). The AMRC do not exist in the cauda (Sinowatz, 1981). The IEM and IEL occur in variable numbers in the neighbouring tubules of the same section (Goyal, 1985).

2.5.4 Extraepithelial, peritubular and interstitial structures

In bulls, the BL of the epididymal epithelium is distinctly developed along the entire length of the duct. A large number of hemi-desmosomes exist between the BC and the BL. Subepithelial capillaries often appear as they are taken inside epithelial folds. At the level of the light microscopy, this give rises to the wrong impression that these capillaries are intraepithelially located. With the electron microscope it becomes obvious that they still always separated from the epithelium through the BL (Sinowatz, 1981). Peripheral to the

BL, a weak layer of CT consisting of fibroblasts and collagen fibrils are longitudinally extended. Usually, a definite line of demarcation between the collagen fibrils and the smooth muscle cells of the luminal wall is seen. The amount of the smooth muscle cells around the epithelium increases continuously from the first segment through the fifth. The mean thickness of the muscular layer of the first segment reaches 20 µm and that of the fifth reaches 40 µm. The cauda epididymidis characterized by a markedly thick (over 80 µm) muscular wall (Sinowatz, 1981). The single smooth muscle cells show only minimal variation in their form between the different segments of the epididymal duct. However, those predominating in the caput and the corpus have a spindle shaped contour, whereas the smooth muscle cells of the cauda have truncated ends. A direct contact between the neighbouring muscle cells by nexus is rarely seen. A definite BL surrounds these cells and additionally each cell is separated from the neighbouring cells through a layer of collagenous fibrils. Elastic material has been not observed between the muscle cells or under the epithelial BL. Muscle cells have elongated nuclei, which adapt themselves completely to the narrow cell bodies leaving a narrow cytoplasmic rim peripherally. The predominant components of the cytoplasm are myofilaments, which originate from thickenings of the cell membrane and run in bundles obliquely or parallel to the longitudinal axis of the cell. Micropinocytotic vesicles are seen near to sites of the cell membrane that are devoid of the membrane thickenings. The mitochondria are of the cristae type and have an elongated or oval shape. They exist in a large number and occur mainly at the poles of the nucleus. The muscle cells contain a moderately developed Golgi apparatus as well as some strands of rER. Many glycogen granules are found throughout the cytoplasm (Sinowatz, 1981).

Interstitial spaces are occupied by loose CT with the blood and lymph vessels as well as nerve fibres. The cellular elements of this CT are, in addition to the fibroblasts and macrophages, granulocytes, lymphocytes and mast cells (Sinowatz, 1981; Goyal, 1985). The collagen fibres of the interstitium are loose and randomly arranged (Sinowatz, 1981).
The epithelial cells of the canine epididymis are surrounded basally by a thick well-defined BL. Micropinocytosis is only evident at the base of the BC. Beyond the BL there is a well-organized arrangement of collagen fibres, fibroblasts and smooth muscle cells running in several closely packed layers. The intertubular space is occupied by a relatively loose arrangement of collagen fibres and fibroblasts. Blood capillaries are seen quite frequently, particularly near the BC (Chandler et al., 1981).

2.6 Epididymal physiology

Spermatozoa undergo many physicochemical changes between the rete testis and cauda epididymidis, which are collectively described as epididymal sperm maturation (Pineda, 1989). In addition to its roles in sperm transport and maturation, the cauda epididymidis serves as a reservoir for storage of viable spermatozoa (Bedford, 1975; Hamilton, 1975; Brooks, 1983; Pineda, 1989). The cauda epididymidis provides the favourable conditions for this storage through its distant location from the body heat most distally in the scrotum ensuring a relatively lower (32°C) temperature (El Azab, 1966; Pineda, 1989). The solubility of oxygen in a fluid is higher at the lower temperature. Coincidently the oxygen requirement of sperms is reduced. This provides suitable conditions for spermatozoa storage over a long time (Djakiew and Cardullo, 1986). Furthermore, the existence of the high molecular weight epididymal glycoprotein, Immobilin, might inhibit the motility of spermatozoa by creating a viscoelastic epididymal environment for the spermatozoa stored in the epididymal tail. The metabolic activity of sperms is reduced and consequently the utilization of oxygen (Pineda, 1989).

The process of epididymal sperm maturation involves changes in several morphofunctional aspects of the mammalian spermatozoon (Briz et al., 1995). These include:

- Modifications in the patterns of movement.
- Changes in the metabolic patterns and the structural condition of the specific organelles of the tail.
- Changes in the nuclear chromatin and modification of the acrosomal shape.
- Progressive loss of water and increase of the specific gravity.
- Migration of the cytoplasmic droplet through the midpiece and its detachment.
- Modification of the spermatozoa plasma membrane.

The two most important features of the sperm associated with its fertilizing capacity are acquired during epididymal transit are motility and chromatin stability (Rodríguez and Bustos-Obergón, 1994). This stability is acquired through chromatin packing, mostly due to protamine disulfide bond formation (Calvin and Bedford, 1971). The morphological changes in the rat sperm are attributable to the condensation of the nucleus during epididymal transit (Fornés and Bustos-Obergón, 1994). The full progressive motility of the spermatozoa presumably requires two contributing factors (Brooks, 1983). The first factor is a raised level of intracellular cyclic AMP (cAMP), which leads to a vibratory movement of the sperm tail. The second factor is a specific protein of epididymal origin termed “forward motility protein” (FMP); (Brandt et al., 1978). FMP is a glycoprotein found in the bovine seminal

plasma and can induce forward motion in immotile caput spermatozoa under conditions of elevated cAMP levels (Brandt et al., 1978). FMP is of epididymal origin and is not found in other bovine tissues or fluids (Acott et al., 1979) and is presumably synthesized in the proximal corpus region of the bovine epididymis. It is bound tightly to the plasma membrane of the spermatozoa during their transit through this epididymal segment (Acott and Hoskins, 1978). FMP changes the nature of the response of sperms to an elevation in intracellular cAMP levels. Therefore, sperms develop a strong progressive motility instead of the uncoordinated vibratory motion (Acott and Hoskins, 1981). The aforementioned mechanism is found in many mammalian species (Acott et al., 1979).

Testicular sperms are both immotile and infertile (Brooks, 1983). Mammalian spermatozoa undergo many biochemical and morphological changes during their passage through the epididymis (Bedford, 1975; Briz et al., 1995). As the spermatozoa reach the cauda epididymidis, they acquire capacity for both motility and fertility (Hamilton, 1975; Orgebin-Crist et al., 1975). During their epididymal transit, the plasma membranes of spermatozoa become coated with glycoproteins. The latter may play a significant role in fertilization (Faye et al., 1980).

Epididymal sperm maturation is also accompanied by distinct changes in lipid composition (Scott, 1973), energy metabolism (Voglmayr, 1975) and surface characteristics (Bedford, 1975). The membrane surface is presumably engaged with many cellular activities including recognition, adhesion, proliferation, differentiation and motility (Moscona, 1974). Alterations in epididymal sperm membranes may result from the incorporation of protein, sugar and lipids originating from the epididymal fluid (Moore, 1998).

Several types of surface changes take place during the epididymal maturation of spermatozoa (Olson and Danzo, 1981). Mechanisms which contribute to these changes include modifications of pre-existing surface components, unmasking of surface components, de novo synthesis and/or insertion of new surface components and adsorption of macromolecules produced by the extra-testicular excurrent duct system (Orgebin-Crist et al., 1975; Voglmayr et al., 1980; 1982). The alterations of the sperm surface by addition or subtraction of proteins or by modifications of existing proteins leads to the exposure of the correct surface receptors to allow species-specific recognition between sperm and egg which is substantial to the process of fertilization (Brooks, 1983). This change on the sperm surface might be induced by several mechanisms such as enzymatic activities, either intra- or extratesticular enzymes like proteinases and glycosidases, or by the association of new

exogenous compounds on the membrane of spermatozoa, which may be able to mask some other surface polypeptides (Dacheux et al., 1989).

Seminal fluid is absorbed in the ductuli efferentes and caput epididymidis, causing sperm concentration to increase throughout the epididymis (Pineda, 1989). The absorptive capacity of the epididymis may be related to the fact that it develops from the mesonephric duct (Rüsse and Sinowatz, 1991). The spermatocrit steadily increases at more distal sampling (cannulation) points, indicating a progressive removal of fluid (Brooks, 1983). Water removal is probably a consequence of active transport of sodium chloride out of the epididymis (Wong and Yeung, 1978). Sodium ions are replaced in part by potassium ions; however, osmotic pressure is maintained principally by the addition of certain organic molecules, rather than inorganic ions. These organic molecules include glycerylphosphoryl-choline (GPC), carnitin and inositol (Brooks, 1983). GPC is a synthetic product of the epididymis itself, especially in the caput epididymidis (Brooks, 1979). In contrast to GPC, carnitin is not synthesized by the epididymis, but is actively transported from the blood stream (Brooks et al., 1973; Brooks, 1983) through the epididymal epithelium.

The epididymis secretes both chloride and bicarbonate when the cells are stimulated with adrenergic agonists such as adrenaline or nor-adrenaline (Hinton and Palladino, 1995), as well as with vasoconstrictor peptides including endothelin and angiotensin converting enzyme (Wong et al., 1990). A standing osmotic gradient is established across the epididymal epithelium to move water from the lumen into the interstitium (Hinton and Palladino, 1995). This takes place through passive movement of sodium across the apical surface of the epithelium followed by an active transport across the basolateral membrane, presumably via Na^+/ K^+-ATPase. The establishment of a sodium gradient draws chloride and water out of the lumen. This electroneutral movement is probably due to the secretion of both hydrogen and potassium into the lumen (Wong, 1990). However, concentrations of hydrogen and potassium are not sufficient to make up for the loss of sodium chloride (Hinton and Palladino, 1995). Small organic solutes made up the difference to maintain the osmolality of the fluid (Levin and Marsh, 1971). Water movement across the epididymal epithelium is dependent upon androgens (Wong and Yeung, 1977) and aldosterone (Turner and Cesarini, 1983). The epididymal fluid is hyperosmolar to blood plasma (Levin and Marsh, 1971). The hyperosmolar milieu may be a factor in prolonging the survival of spermatozoa during their transit along the epididymal duct (Hinton and Palladino, 1995).

The activities of the epididymal epithelium are androgen-dependent (Orgebin-Crist et al., 1975; Bedford and Hoskins, 1990). Androgens are essential for maintenance of the structural

and functional integrity of the epididymis (Moore and Bedford, 1979a; Pineda, 1989). The epididymal luminal fluid contains a number of steroids, such as testosterone and dihydrotestosterone (DHT) (Turner et al., 1984). The latter is the predominant steroid in epididymal fluid, but unlike in seminiferous tubules, most of androgen is bound to androgen binding protein (ABP) (Turner et al., 1984; Turner, 1991). This may interpret the active endocytosis of ABP by the PC of the proximal epididymal region (Gerard et al., 1988) as a possible pathway through which androgens get access to its target receptors inducing its regulatory effects, such as gene expression (Hinton and Palladino, 1995). A region-specific regulation of gene expression is one of the underlying cellular mechanisms affecting the synthesis and subsequent secretion of proteins by the epididymal epithelium (Cornwall and Hann, 1995). On the contrary, it seems more likely that androgen effects are of a general anabolic nature on whole tissue metabolism, whereas individual gene products are regulated by other specific factors (Uhlenbruck et al., 1993).

The caput epididymidis produces, in an androgen-dependent manner, the enzyme glutathione peroxidase (Jimenez et al., 1990), which associates with the head of spermatozoa and protects sperms against oxidative damage (Perry et al., 1992). Gamma glutamyl transpeptidase (GGT) exists at the apical surface of epididymal epithelial cells as an integral membrane protein and is also found in epididymal fluid with its highest activity in head region. It is involved in protecting spermatozoa from oxidative stress and toxic chemicals (Hinton et al., 1991).

The composition of the epididymal luminal fluid is distinctly different from that of blood plasma, indicating that the two pools are kept in their separate compartments (Hinton and Palladino, 1995). This may be achieved through the blood-epididymis barrier, which is represented by well-developed zonulae occludentes represented by an extensive meshwork along the entire length of the epididymis (Hoffer and Hinton, 1984).

Spermatophagy may account for removal of damaged, aged or surplus spermatozoa. Degenerated spermatozoa are removed partly by phagocytic bodies in the epididymal lumen and possibly by cells of the epididymis themselves (López and Bustos-Obergón, 1995). PC lining the cauda epididymidis may be involved in sperm phagocytosis.
Incorporation of sperm tails into epididymal cells and removal of the sperm remnants through lysosomal activity (Glover, 1969) are evident. Spermatophages were encountered in

the lumen of the proximal region of stallion epididymis (López and Bustos- Obergón, 1995). Occasional sperm phagocytosis was proved in the male genital tract of rhesus monkey and human (Holstein, 1978) and of bull (Goyal, 1982) by the existence of the intraluminal macrophages.

3 MATERIAL AND METHODS

3.1 Materials

3.1.1 Animals

3.1.1.1 Adult specimens

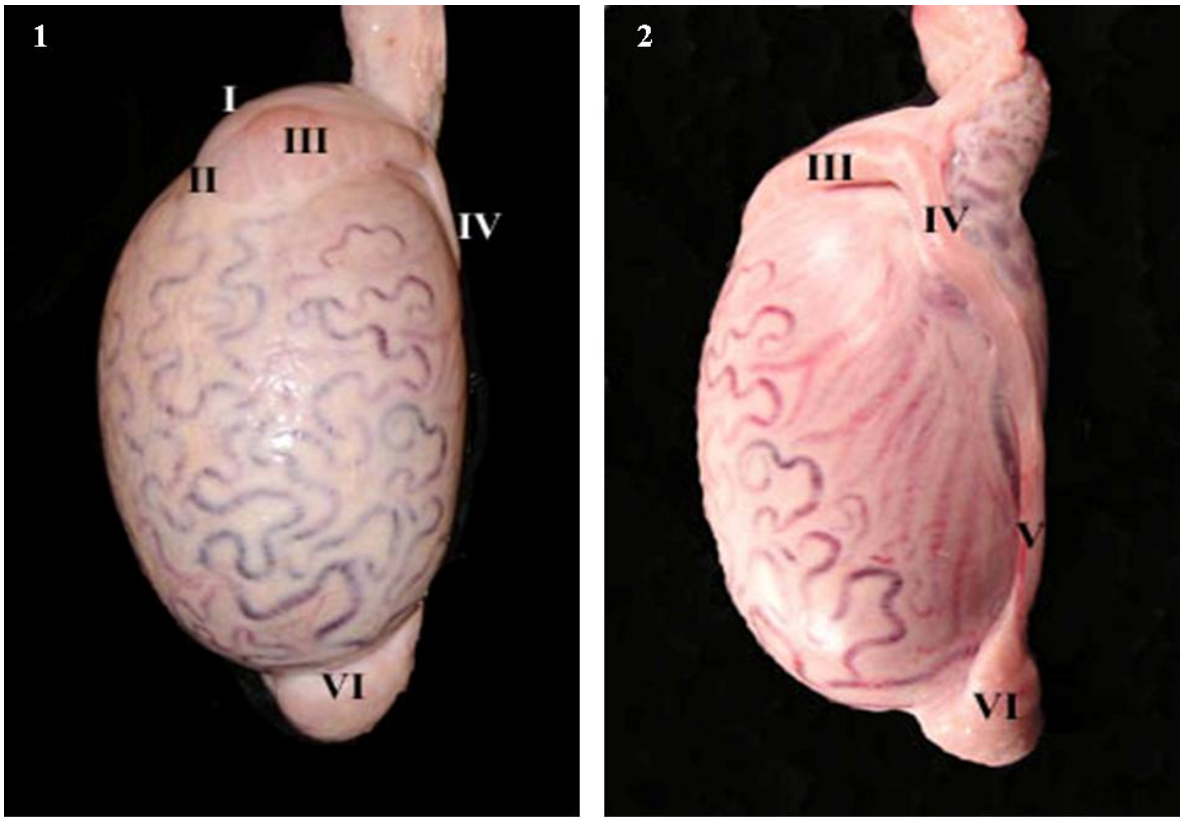


Fig. 1 and 2: Different segments of adult bovine epididymal duct. Segments I, II and III constitute together caput epididymidis, segments IV and V correspond to corpus epididymidis, segment VI represents cauda epididymidis (Nicander, 1958).

Bovine epididymal specimens were obtained from 21 adult clinically healthy bulls (Deutsches Fleckvieh) slaughtered in the abbatoir of Munich, Germany. All the specimens were taken directly after slaughter. The epididymis from each animal was divided into 7 portions; the most proximal one represents the efferent ductules. Distal to the latter and according to Nicander (1958) the epididymal duct was divided into six segments (Fig. 1 and 2). The first three segments constitute the head region; the 4th and the 5th correspond to the body whereas the 6th segment represents the tail region. Additional specimens were wrapped

in aluminium foil and shock frozen in liquid nitrogen. They were stored at -70 °C until sectioned using cryostat.

3.1.1.2 Foetal specimens

The bovine male foetuses were collected from the slaughtered pregnant cows in the slaughterhouse in Munich. Their ages were estimated according to the crown rump length (CRL) as described by Evans and Sack (1973), Rüsse and Sinowatz (1991). The collected material was summarized in table 3.

Table 3: Ages (pcd) of the foetal specimens.

CRL (cm)	PCD	Gestation Month	Number of foetuses
10	75	3	3
13	80	3	4
18	95	4	3
20	100	4	3
24	110	4	3
30	130	5	4
36	140	5	2
56	185	7	2
63	210	7	3
90	285	9	1

CRL = crown rump length, pcd = postcoital day.

3.2 Methods

3.2.1 Light microscopy

3.2.1.1 Fixation, Processing and Sectioning

Specimens from 15 adult bulls were fixed in different solutions of fixatives for routine histological staining, glycohistochemistry and immunohistochemistry. Additionally, specimens from 3 adult bulls were used for frozen sections to elucidate the effect of different methods of fixation on the pattern of the staining reactivity to lectins.

3.2.1.1.1 Paraffin sections

0.5 cm-thick specimens were fixed for 24 hours in Bouin's fluid (Romeis, 1989), in 7% buffered formalin solution and in methanol: glacial acetic acid (2:1) mixture. They were then

preserved in 70% ethanol until embedding. The specimens were then dehydrated in a graded series of alcohol and embedded automatically by using a Shandon-Duplex-Processor. Finally the prepared tissue specimens were embedded in paraplast blocks. Sections were cut using a Leitz rotatory microtome (type 1516) at 5µm.

3.2.1.1.2 Frozen sections

The shock frozen blocks were placed in the -20 °C cryostat for at least one hour before sectioning. The blocks were then cut at 7 µm thick using Reichert-Jung cryostat (2800 Frigocut N). Sections were thaw mounted onto clean poly-L-lysine-coated glass slides. The frozen sections were left at room temperature for 30 minutes and then immersed in cold acetone (4°C) for 10 minutes and then left to dry in air at room temperature for 30 minutes before being kept in the -70°C deep freeze until staining.

3.2.1.2 Histological Staining

3.2.1.2.1 Conventional staining

Sections were deparaffinized in xylene, then rehydrated in descending grades of ethanol down to distilled water. After staining they were dehydrated in ascending grades of ethanol, cleared in xylene and covered with Eukit® (Romeis, 1989). Different staining procedures were carried out: Haematoxylin and Eosin staining (Harris, 1898) modified according to Romeis (1989) for demonstration of the general histological structure of the examined bovine epididymis; Periodic Acid Schiff (PAS) reaction (Drury and Wallington, 1980) after McManus, (1948) and PAS reaction after amylase digestion; Alcian blue 8 G X (pH 2.5) and (pH 1.0) after Steedman, (1950) and Toluidin blue for detection of acid mucopolysaccharides. Trichrom staining according to Goldner, (1938), Resorcin-fuchsin-Nuclear red (Elastic stain) after Weigert, (1898) for detection of elastic fibers, and van Gieson's method, (1889), after Gabe, (1976). The stained sections were examined using Leitz Dialux 20 Microscope. Photos were taken by using Agfa Pan 25 ASA, Kodak, Tri-X pan, 400ASA, Kodak, or Ectachrome plastic, 64 ASA films.

3.2.1.2.2 Lectin histochemistry (Glycohistochemistry)

Detection of the glycoconjugates was carried out through using Fluoresceinisothiocyanate (FITC)-labelled lectins (Sigma; Munich) the studied lectins are listed in (Table 4).

Table 4: FITC-lectins used in glycohistochemistry.

Lectin	Origin	Carbohydrate specificity
GSA-I (*Griffonia simplicifolia* agglutinin I)	*Griffonia simplicifolia*	Galactose (α-GalNAc> α-Gal)
PNA (peanut agglutinin)	*Arachis hypogeae* (peanut)	Galactose (Galβ1,3GalNAc> α- and β-Gal)
ECA (*Erythrina cristagalli* agglutinin)	*Erythrina cristagalli*	Galactose (Gal(β1,4)-GalNAc
WGA (Wheat germ agglutinin)	*Triticum vulgare* (Wheat germ)	N-Acetylglucosamine (GlcNAc-[(β1,4)-GlcNAc]$_{1-2}$> [β-GlcNAc]$_n$>Neu5Ac)
Con A (Concanavalin A)	*Canavalia ensiformis* (Jack bean)	Mannose (α/β-Man>α-GLc>α-GLcNAc)
LCA (*Lens culinaris* agglutinin)	*Lens culinaris* (lentil)	α-D-Man, α-D-Glc
PSA (*Pisum sativum* agglutinin)	*Pisum sativum* (garden pea)	α-D-Man, α-D-Glc
DBA (*Dolichos biflorus* agglutinin)	*Dolichos biflorus* (horse gram)	N-Acetylgalactosemin (GalNAcα1,3GalNAc>αGalNAc)
HPA (*Helix pomatia* agglutinin)	*Helix pomatia* (edible snail)	N-Acetylgalactosemin (GalNAcα1,3GalNAc>αGalNAc)
SBA (soybean agglutinin)	*Glycine maximus* (Soybean)	N-Acetylgalactosemin (α-GalNAc>β-GalNAc>α-/β-Gal)
VVA (*Vicia villosa* agglutinin)	*Vicia villosa* (hairy vetch)	N-Acetylgalactosamine (GalNAc α1,3Gal = αGalNAc αGalNAc)
LTA (*Lotus tetragonolobus* agglutinin)	*Tetragonolobus purpureas* (*Lotus Asparagus* pea)	L-Fucose (α-L-Fuc)
UEA-I (*Ulex europaeus* agglutinin I)	*Ulex europaeus* (gorse seed)	L-Fucose (α-L-Fuc)

Deparaffinization was performed in xylene for 20 minutes, rehydration through descending grades of ethanol until distilled water for 5 minutes each (frozen sections were directly immersed in distilled water for 5 minutes). Sections were then washed with 0.05 M Tris buffered saline (TBS) at pH 6.8 for 30 minutes at room temperature. The sections were incubated for 12 hours at 4°C with FITC-conjugated lectins listed in table 4. The lectin concentration was 33µg/ml TBS. After incubation the sections were washed with TBS three times and covered with polyvinyl alcohol and ethylenglycol in TBS at pH 8.5 (Serva,

Heidelberg). The control group was incubated with TBS only. Another group of sections was incubated with different FITC-conjugated lectins and their specific inhibitory sugars to confirm the specificity of the results. The sections were studied using a Leitz Dialux 20 microscope. The photos were taken by using Kodak T maxfilm 400.

3.2.1.2.3 Immunohistochemical staining

The immunohistochemical studies were performed using the Avidin-Biotin-peroxidase Complex (ABC) method according to Hsu et al., (1981). Sections were deparaffinized in xylene for 20 minutes, rehydrated in descending grades of alcohol until distilled water.
Sections were then washed three times in a phosphate buffer saline (PBS), pH 7.4 for 5 minutes each. Endogenous peroxidase was inhibited with 1% H_2O_2 for 10 minutes. After intense washing under current tap water for 10 minutes, and washing in PBS (three times, 5 minutes each), the sections were covered with DAKO protein block serum-free (DAKO, Hamburg, Germany) for 10 minutes at room temperature. They were then incubated with the specific primary antibody (Table 5) for 12 hours at 4ºC.

However, for detection of certain proteins (FGF-1, connexin) it is necessary to unmask the antigen through heating the sections in citrate buffer (pH 6.00) three times (10 minutes each) in microwave before adding the primary antibody. For antigen retrieval (laminin and the macrophage marker: CD 68), sections were pretreated with 0.1% protease for 5 minutes before adding the primary antibody.

After an overnight-incubation with the primary antibody and washing the sections with PBS three times (5 minutes each), incubation with a secondary antibody (Table 5) for 30 minutes at room temperature was performed.

After washing the sections in PBS (three times 5 minutes each), they were incubated with streptavidin-biotin horseradish peroxidase complex (DAKO, Hamburg, Germany) for 30 minutes at room temperature.

The reaction was developed using diaminobenzidin (DAB) solution for 10 minutes at room temperature. Counter-staining of the nuclei was done with Haematoxylin for 30 seconds. Sections were dehydrated through ascending grades of ethanol, cleared with xylene, and covered with DPX.

Table 5: Antibodies used in immunohistochemistry.

Primary antibody against	**Dilution of Prim. Ab.**	**Origin of Prim. Ab.**	**Secondary Antibody**	**Dilution of Sec Ab.**	**Source of Prim. Antibody.**
S100	1:400	Rabbit	Antirabbit, IgG Biotin from pigs (DAKO, Hamburg)	1: 300	(DAKO, Hamburg)
FGF-1	1:1000	Rabbit	Antirabbit, IgG Biotin from pigs (DAKO, Hamburg)	1: 300	Prof. Dr Schams, TU-München in Weihenstephan.
FGF-2	1: 1000	Rabbit	Antirabbit, IgG Biotin from pigs (Dako, Hamburg)	1: 300	Prof. Dr Schams, TU-München in Weihenstephan.
Connexin 43	1: 400	Mouse	Antimouse IgG Biotin from Rabbit (DAKO, Hamburg)	1: 300	BD, Bioscinces, Heidelberg
Laminin	1: 500	Rabbit	Antirabbit IgG Biotin from pigs (DAKO, Hamburg)	1:300	Serotec, Düsseldorf
Galactosyl-transferase	1: 500	Chicken	Antichicken IgG Biotin from Rabbit (Rockland, USA)	1:400	Institute of Vet. Anatomy II, LMU-München
VEGF	1: 800	Rabbit	Antirabbit IgG Biotin from pigs (DAKO, Hamburg)	1:300	DAKO, Hamburg
ACE	1: 500	Chicken	Antichicken IgG Biotin from Rabbit (Rockland, USA)	1:400	Institute of Vet. Anatomy II, LMU-München
α-SMA	1: 200	Mouse	Antimouse IgG Biotin from rabbit (DAKO, Hamburg)	1: 300	DAKO, Hamburg
CD4 (CC30)	1: 200	Mouse	Antimouse IgG Biotin from rabbit (DAKO, Hamburg)	1: 300	Serotec, Düsseldorf
CD8 (CC58)	1: 50	Mouse	Antimouse IgG Biotin from Rabbit (DAKO, Hamburg)	1: 300	Serotec, Düsseldorf
CD68 (EBM 11)	1: 50	Mouse	Antimouse IgG Biotin from Rabbit (DAKO, Hamburg)	1:300	DAKO, Hamburg

3.2.2 Transmission electron microscopy

Small tissue blocks of about 1-2 mm side length were taken from the efferent ductules and the different epididymal segments and fixed in Karnovsky' solution (2.5% formaldehyde–2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 mixture) for 4 hours at 4°C. After that the specimens were washed in 0.1 M sodium cacodylate buffer at pH 7.4 for 24 hours. The specimens were dehydrated in graded series of alcohols and embedded in Epon (Polyscience, Eppelheim, Germany). Semithin sections (1 µm) were cut using an ultramicrotome (Ultratome Nova, LKB, Bromma, Switzerland) and stained with methylene blue-azur II (Richardson et al., 1960). Ultra thin sections (60 nm) were cut on a Reichert ultramicrotome, mounted on copper grids and contrasted with uranyl acetate and lead citrate. They were examined and photographed using a Zeiss EM 902 electron microscope.

3.3 Chemicals

- **Bouin's solution**

750 ml	saturated solution of picric acid
250 ml	formaldehyde
50 ml	glacial acetic acid

- **Cacodylate Buffer**

a) Solution A

$Na(CH_3)2AsO_23H_2O$	8.56 g
(Polysciences Inc., Warrington/USA)	
Distilled water	200 ml

b) Solution B

HCl, 0, 2 (Merck, Darmstadt)

for Cacodylate buffer, 0, 2 M, pH 7.2:

Solution A	50 ml
Solution B	4.2 ml
Distilled water	100 ml

for Cacodylate buffer, 0,1 M, pH 7.2:

0.2 M solution	50 ml
Distilled water	100 ml

- **Contrasting solution for electron microscopy**

  OsO_4, 4% 2 ml
  (Polysciences Inc., Warrington/USA)
  Distilled water 6 ml
  Potassiumferrocyanide 0.12 g
  (Sigma, Deisenhofen)

- **DAB preparation**

  Solution A: 50 ml TBS buffer + 0.15 ml 30% H_2O_2
  Solution B: 60 ml PBS + 3 DAB tablets
  0.4 ml of solution A are mixed with solution B. Filtration of the mixture

- **Karnovsky solution (Karnovsky, 1965)**

  Paraformaldehyde 10 g
  Distilled water 100 ml
  NaOH, 1M 6 drops

- **PBS Buffer (phosphate buffered saline)**

  42.5 g Sodium chloride
  6.35 g Disodiumhydrogenphosphate-dihydrate
  1.95 g Sodiumdihydrogenphosphate-monohydrate in 5 liters distilled Water at pH 7.4 to 7.6.

4 RESULTS

4.1 Prenatal development of the bovine epididymis

4.1.1 Microscopic anatomy of the bovine prenatal epididymis

In this study, the samples collected for the study of prenatal development of the bovine epididymis comprised foetuses with 10 cm CRL until full-term (90 cm CRL). On the basis of the crown rump length (CRL), the samples studied in this work are categorized in five stages:

4.1.1.1 The third gestational month

The prenatal development of the bovine epididymis in foetuses of 10 to 13 cm CRL (75 to 80 pcd) is mainly confined to establishment of the urogenital junction when the extratesticular rete testis (ETRT) becomes patent and is connected to the mesonephric duct via the efferent ductules (Fig. 3) derived from the giant mesonephric corpuscle (GMC). Growing efferent ductules are seen joining the ETRT and take their way towards the mesonephric duct (Fig. 3). The efferent ductules are lined by a simple low columnar epithelium resting on a well-distinct PAS-positive basal membrane. The epithelial cells possess large ovoid vesicular nuclei which contain one or more prominent nucleoli. The nuclei are in close contact with the basal lamina and occupy most of the cell leaving a narrow apical cytoplasmic area. Some nuclei are apically located and sometimes show mitotic figures. Some of the cells lining the efferent ductules are provided with cilia. The periductal mesenchymal cells are concentrically arranged around the ductules in about 4 cell layers with their cytoplasmic processes joining each other and leaving wide intercellular spaces or lacunae among them. The nuclei of these cells are large elongated and less vesicular than those of the epithelium and possess prominent nucleoli. The interstitium is highly cellular containing comparatively large mesenchymal cells similar to the peritubular ones; however they present nuclei of various forms and are irregularly distributed. They join each other through long cytoplasmic processes leaving wider intercellular spaces in-between. Blood vessels are found in the interstitium and some of them are filled with blood cells. The primordium of the epididymal duct is represented at this level by a single cross-cut mesonephric duct. The latter can be easily differentiated from the efferent ductules through its wider lumen and the thicker concentric array of the peritubular mesenchymal cells. Mesonephric duct is lined by a simple cuboidal or low columnar epithelium resting on a well-distinct PAS-positive basal membrane. The epithelial cells

possess large, ovoid nuclei with one or more nucleoli. The nuclei mostly occupy the basal tow-thirds of the cells. The peritubular mesenchymal cells are similar to those surrounding the efferent ductules however they form many more layers (7 to 15 cell layers). The mesonephric duct then precedes distalwards parallel to long axis of the testis in a straight course to the site of the cauda epididymidis (Fig. 3). At the same time both the Müllerian duct and the mesonephric tubules undergo regressive changes.

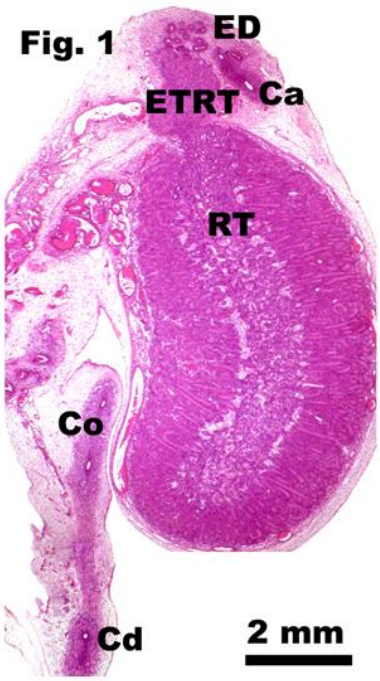


Fig. 3: H&E-stained section from a bovine foetus with 13 cm CRL (80 pcd). Establishment of urogenital junction. RT = rete testis, ETRT = extratesticular rete testis, ED = efferent ductules, Ca = caput, Co = corpus and Cd = cauda epididymidis.

4.1.1.2 The fourth gestational month

At the foetal age of 95 to 100 pcd (CRL 18 to 20 cm), the efferent ductules are lined by a simple columnar epithelium. The nuclei of the epithelial cells are large, mainly elliptical with one or more prominent nucleoli. Some of them abut directly on the basal cell membrane, while the others are located at somewhat higher levels showing mitotic activity. Some cells are equipped with kinocilia. The epididymal duct is represented by the mesonephric duct which slightly coils in the head region. However it still runs in a straight course in the areas corresponding to the corpus and cauda epididymidis. The duct is lined by a simple cuboidal or low columnar epithelium equipped with stereocilia and resting on a well-distinct PAS-positive basal membrane. The epithelial cells have large ovoid nuclei with one or more

prominent nucleoli. These nuclei abut mainly on the basal plasma membrane, occupy the basal two-thirds of the cells and leave only a moderate supranuclear region of cytoplasm. The duct is surrounded by about 7 layers of the concentrically arranged mesenchymal cells.

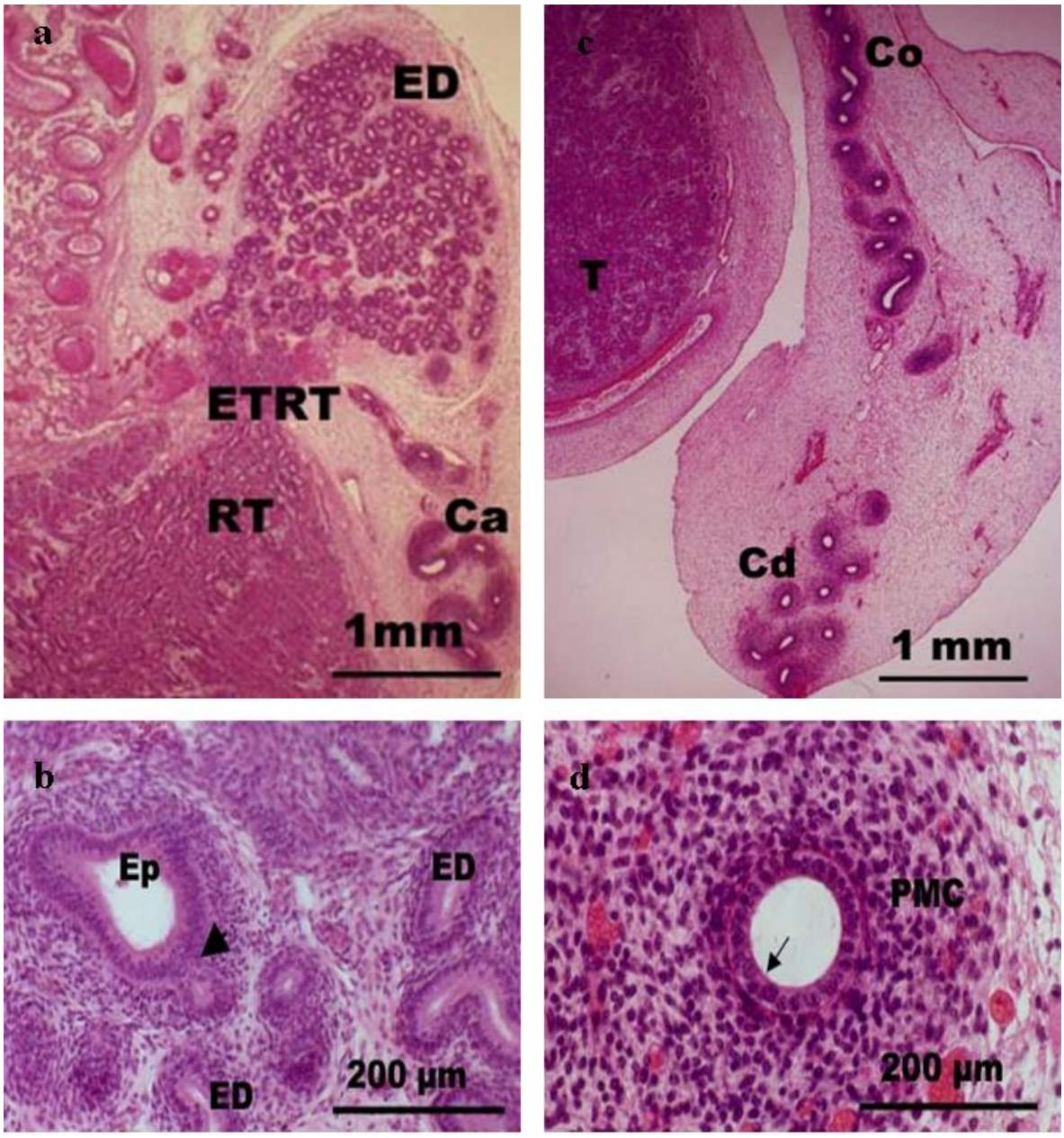


Fig. 4: H&E-stained caput (a and b), corpus (c) and cauda (d) epididymidis from a bovine foetus of 24 cm CRL . RT = rete testis, ETRT = extratesticular rete testis, ED = efferent ductules, Ep = epididymal duct, T = testis, Ca = caput epididymidis, Co = corpus epididymidis, Cd = cauda epididymidis, PMC = peritubular muscle coat, stereocilia = arrow (d). Arrow head (b) points to joining of an efferent ductule to epididymal duct .

The interstitium is highly cellular and the mesenchymal cells are large and heteromorphic possessing large vesicular nuclei and long cytoplasmic processes. Both the mesonephric

tubules and the Müllerian duct are seen undergoing regression. At the foetal age of 110 pcd (24 cm CRL), the efferent ductules undergo strong coiling as indicated from the high density of numerous profiles of the ductules (Fig. 4a). Furthermore, a connection between a ductule and the epididymal duct is found (Fig. 4b). The epithelium lining ED is similar to that of the former stage. Some epithelial cells are ciliated and the apical surface of some cells present eosinophilic granules. The ductules are surrounded by 3 to 4 cell layers of somewhat differentiated mesenchymal cells in their way to form periductular smooth muscle investment. The interstitium is still highly cellular and expresses some differentiation and deposition of extracellular matrix. The intercellular spaces become fewer and narrower than in the former stages. Epididymal duct begins to coil forming few turns along the corpus region and somewhat more turns in the cauda region (Fig. 4c). The convolution, however, is much more marked in the duct at the caput region (Fig. 4a). The epithelium lining the duct in the epididymal head (Fig. 4b) and body is higher than that of the tail (Fig. 4d). The nuclei of the epithelium lining the caput and corpus epididymidis are in general long oval, less vesicular and have one or more nucleoli. Most of these nuclei abut on the basal cell membrane, however some spherical nuclei are seen near from the apical surface where mitotic figures may be found. The apical surface of epithelium at head region is provided with stereocilia and presents eosinophilic granules.The epithelium lining the cauda epididymidis is shorter and possesses large ovoid to round nuclei. The latter are less vesicular with one or more nucleoli and some of them express mitotic activity. Most of nuclei are found basally, however some of them are located more apically. The peritubular mesenchymal cells are concentrically arranged in about 10 to 15 layers and those near the epithelial basal membrane differentiate and acquire appearance similar to that of the smooth muscle cells, whereas the outermost ones are still less differentiated.

4.1.1.3 The fifth gestational month

At the foetal age of 130 to 140 pcd (CRL 30 to 36 cm), the efferent ductules are organized in lobules which are separated from each other by connective tissue septa. ED are lined by a simple epithelium consisting of large pyramidal cells, some of which are ciliated (Fig. 5). The periductular cells are arranged in 2 to 3 layers and their nuclei are darker and smaller than before and express the morphology of smooth muscle cells. The epididymal duct in the head region is formed of low columnar simple epithelium (Fig. 5) resting on a well-distinct PAS-positive basal membrane. The apical cytoplasm shows eosinophilic granules and the apical surface is equipped with stereocilia and sometimes presents eosinophilic secretory granules.

The number of the peritubular layers of the differentiated mesenchymal cells reaches about 7 layers (Fig. 5). In the body region the nuclei are more elongated and relatively smaller and occupy the middle of the cells. The apical cytoplasm presents eosinophilic granules and the apical surface is provided with stereocilia. The epithelium in the tail region is the shortest in comparison to the other two regions and is formed of cuboidal to low columnar cells. The nuclei are large ovoid or round and occupy most of the cell.

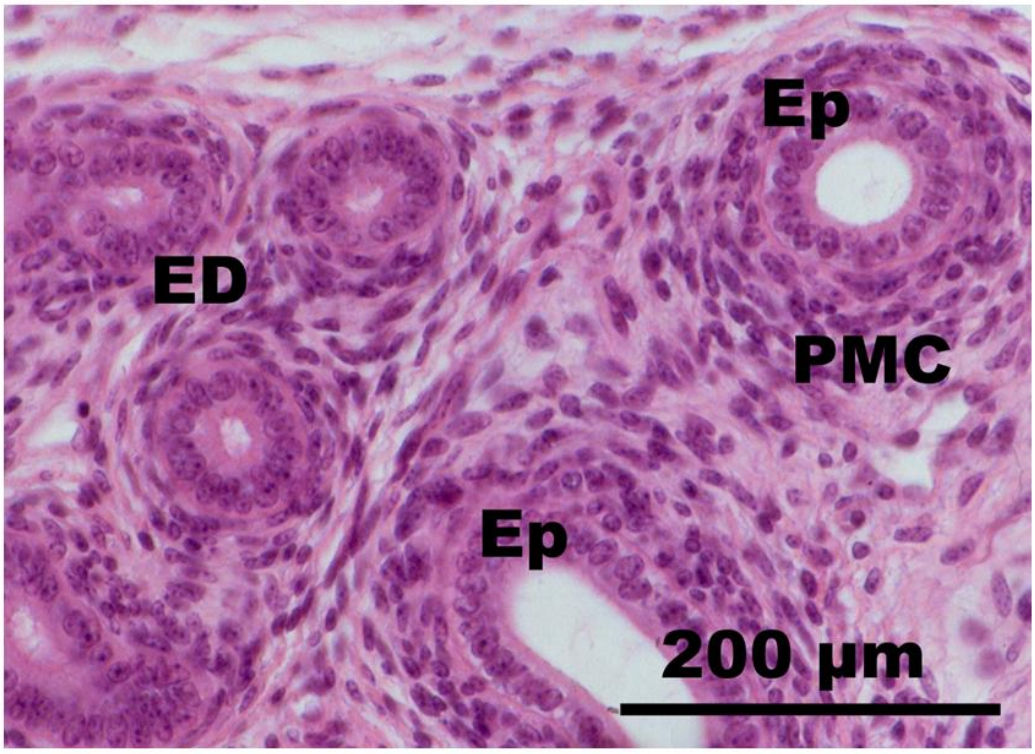


Fig. 5: Head region of H&E-stained caput epididymidis from a bovine foetus of 36 cm CRL (140 pcd). ED = efferent ductules, Ep = epididymal duct, PMC = peritubular muscle coat.

4.1.1.4 The seventh gestational month

At the foetal age of 185 pcd (CRL 56 cm), the efferent ductules are arranged in lobules which are separated from each other by connective tissue septa. They are lined by large pyramidal cells, which are higher than those lining the neighbouring epididymal duct in the head region (Fig. 6a). These cells possess large ovoid or elongated nuclei with one or more nucleoli. Some nuclei are located apically, however, the most of them are basally located leaving a marked supranuclear cytoplasmic area. The apical cytoplasm is eosinophilic and the apical surface presents large eosinophilic granules. The apical surface of many cells is equipped with kinocilia. The lumina of some ductules contain eosinophilic materials. The ductules are surrounded by 2 to 3 layers of differentiated smooth muscle cells (Fig. 6a).

The loosely arranged connective tissue separates between the individual ductules in a lobule as well as between the neighbouring lobules of the efferent ductules. The epididymal duct in the head region next to the efferent ductules is lined by simple cuboidal epithelium (Fig. 6a). The nuclei are large ovoid vesicular and are located nearly at the middle of the cells. The apical surface of some cells is provided with stereocilia. The peritubular smooth muscle cells are arranged in about 5 cell layers (Fig. 6a). The more distal area of the head region is lined by somewhat higher columnar epithelium and the nuclei are large ovoid to elongated oval. The apical surface is equipped with stereocilia and the apical cytoplasm exhibits large eosinophilic granules, which may be seen on the apical surface. Furthermore, eosinophilic materials are seen in the lumina of some tubules. The epididymal duct in the body region is characterized by simple columnar epithelium with large ovoid to elongated vesicular nuclei. They are mostly apically displaced leaving a narrow apical cytoplasm and a wide infranuclear area. However, some nuclei are located in the middle of the cells or more basally. The apical cytoplasm is highly eosinophilic and contains large eosinophilic granules. The apical surface of cells is provided with stereocilia. The epithelium lining the duct in the tail region is simple columnar (Fig. 6b). The apical cytoplasm is highly eosinophilic and contains large, slightly eosinophilic granules. The nuclei of these cells are large oval to narrow elongated and possess one or more nucleoli and some of them may express mitotic activity. They are mostly apically displaced or are located in the middle of the cells; however some of them abut on the basal cell membrane. The infranuclear area is wide and pale (Fig. 6b). The peritubular muscle cells are arranged in about 10 cell layers.

At the foetal age of 210 pcd (CRL 63 cm), the efferent ductules are lined by simple columnar epithelium with ciliated cells. The ductular epithelium rests on a well-distinct PAS-positive basal membrane (Fig. 6c) and is surrounded by a thin lamina propria and about 2 to 3 layers of smooth muscle cells. The supranuclear cytoplasm of some cells presented PAS-positive granules (Fig. 6c). The epithelial cells possess elongated oval nuclei with one or more nucleoli. The nuclei are located in the basal half of the cells leaving a wide supranuclear eosinophilic cytoplasmic area. The nuclei of the ciliated cells are apically displaced (Fig. 6c). The lumina of some ductules contain eosinophilic materials. The epididymal duct in the head region is lined by simple columnar epithelium with stereocilia. The epididymal epithelium rests on a well-distinct PAS-positive basal membrane and the duct is surrounded by a thin lamina propria and about 5 layers of smooth muscle cells. The epididymal duct in the body region is strongly convoluted (Fig. 6d) forming lobules, which are separated from each other through connective tissue septa. It is lined by a simple columnar epithelium with stereocilia.

The supranuclear cytoplasm is highly eosinophilic. The lumina of some tubules contain eosinophilic material. The peritubular muscle cells are arranged in 6 to 8 concentrically oriented cell layers (Fig. 6d). At the tail region the epididymal duct is strongly convoluted forming several lobules which are separated from each other by connective tissue septa. The duct is lined by a low simple columnar epithelium with stereocilia. The epithelium rests on a well-distinct PAS-positive basal membrane and is surrounded by a thin lamina propria and about 10 cell layers of smooth muscle cells.

4.1.1.5 The ninth gestational month

At foetal age of 285 pcd (CRL, 90 cm), ED are highly convoluted and form several groups of lobules, which are separated from each other by connective tissue septa (Fig. 7a). The ductules are lined by a simple columnar epithelium with ciliated cells. The epithelium rests on a distinct basal membrane and is surrounded by a thin lamina propria and 2 to 3 layers of smooth muscle cells. The epididymal duct in the head region is highly convoluted forming many lobules (Fig. 7a). The duct is lined by a simple columnar epithelium with stereocilia (Fig. 7b). The height of the epithelium lining the duct is nearly as long as, or slightly higher than that of the efferent ductules. Some tubules contain eosinophilic material in their lumina. The peritubular smooth muscle cells are concentrically arranged in about 5 cell layers. The interstitium demonstrates differentiated fibroblasts and different types of leucocytes as well as connective tissue fibres. It harbours the epididymal blood vessels and nerves. The nuclei of the cells of the epididymal epithelium are large ovoid in shape and possess one or more nucleoli. Some nuclei express mitotic activity. In general, the nuclei occupy the middle area of the cells leaving only narrow supra- and infranuclear zones of cytoplasm. The supranuclear cytoplasmic zone is highly eosinophilic and the apical surface presents eosinophilic granules. Furthermore, the lumina of some tubules possess eosinophilic material (Fig.7b). In the body region the epididymal duct is markedly convoluted. The duct in this region is lined by a simple columnar epithelium with stereocilia (Fig. 7c). These cells possess large oval nuclei with one or more nucleoli. Most of the nuclei are also located in the middle of the cells, however some are displaced either apical or close to the basal cell membrane. Some cells express mitotic activity especially in the apical area. The supranuclear cytoplasm is highly eosinophilic and contains eosinophilic vesicles, which may be seen on the apical surface of the cells or even in the lumina of some tubules. The peritubular smooth muscle cells are arranged in about 5 cell layers (Fig. 7c) and are separated from the epithelium through a thin lamina propria.

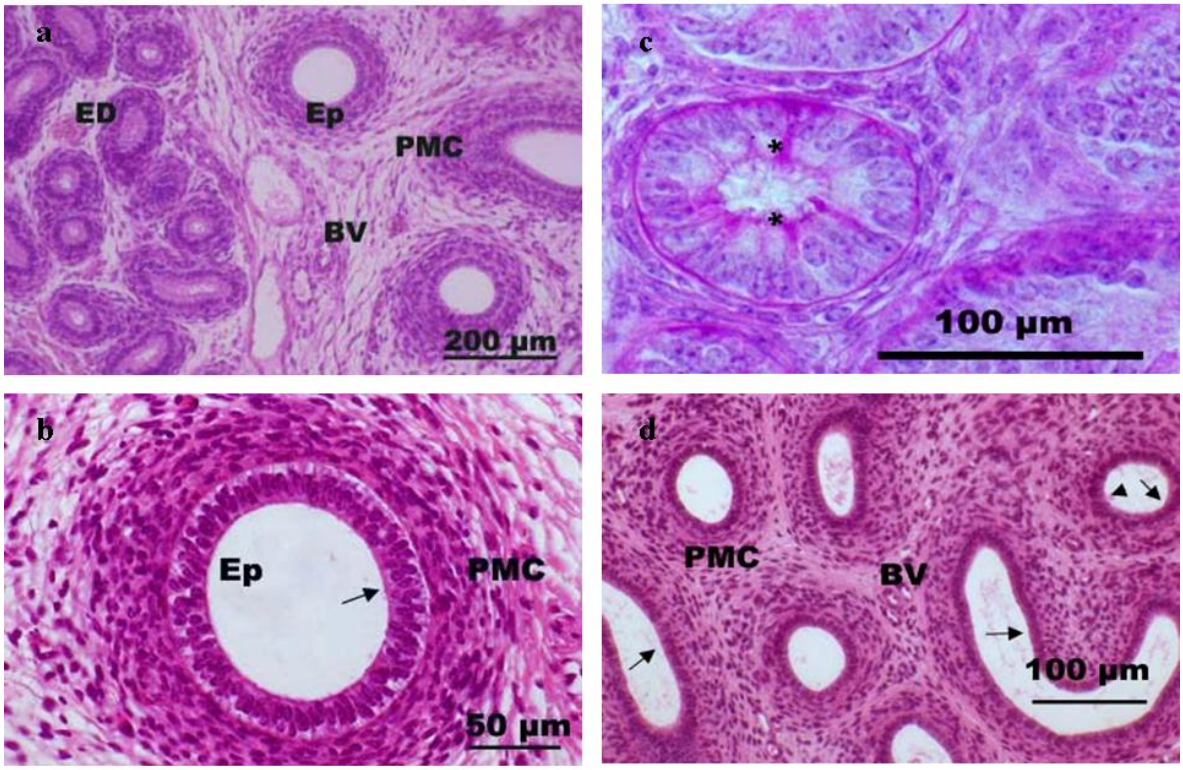


Fig. 6: Caput (a) and cauda (b) of H&E-stained epididymis from a bovine foetus with 56 cm CRL; PAS-stained efferent ductules (c) from a foetus with 63 cm CRL; H&E-stained corpus epididymidis (d) from a foetus with 63 cm CRL. ED = efferent ductules, Ep =epididymal duct, PMC = peritubular muscle coat, BV = blood vessels, arrows point to stereocilia, arrow head points to apical granule, asterisk points to PAS-positive apical cytoplasm.

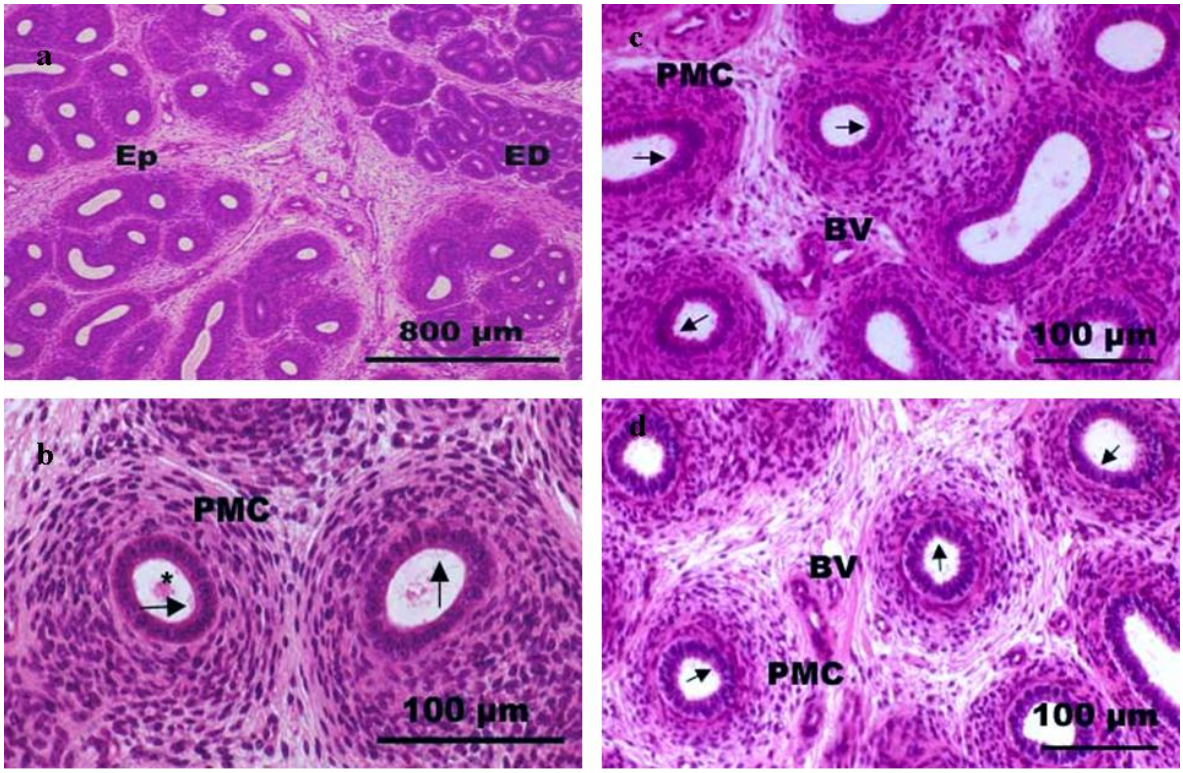


Fig. 7: H & E-stained head region (a) of the epididymis of a full-term (285 pcd, CRL 90 cm) bovine foetus. H & E-stained epididymal duct at the head (b), body (c) and tail (d) regions (CRL 90 cm). ED =efferent ductules, Ep = epididymal duct, PMC = peritubular muscle coat, BV = blood vessels, stereocilia = arrow, luminal secretory material = asterisk.

In the tail region the epididymal duct is strongly convoluted forming many lobules. The duct is lined by a simple columnar epithelium with stereocilia (Fig. 7d). These cells are characterized by large elongated nuclei with one or more nucleoli. The basal cytoplasmic area is pale-stained (Fig. 7d), whereas the apical one is markedly eosinophilic. The peritubular smooth muscle cells are arranged in about 8 to 10 layers and are separated from the overlying epithelium through a thin lamina propria.

4.1.2 Lectin-binding sites of bovine prenatal epididymis (Table 6)

Among the ten lectins (HPA, VVA, UEA-I, LTA, PSA, DBA, LCA, WGA, PNA and GSA-I) used, only three lectins (WGA, PNA and GSA-I) were positive. WGA-binding sites were restricted to the apical surface of the epithelium of the efferent ductules (Fig. 8a). The reaction was evident from CRL 36 cm (140 pcd, 5th gestational month) and upwards. Connective tissue and periductal cells were also WGA-positive.

Table 6: Binding sites of FITC-lectins in foetal efferent ductules and epididymal duct.

Lectin	**CRL (cm)**	**Efferent ductules**				**Epididymal duct**			
		E	**CT**	**PMC**	**BV**	**E**	**CT**	**PMC**	**BV**
WGA	10 to 30 36 to 90	- ++	- +	- ++	- -	- -	- +	- ++	- -
PNA	10 to 90	++	-	-	-	-	-	-	-
GSA-I	10 to 90	++	-	-	++	-	-	-	++
HPA	10 to 90	-	-	-	-	-	-	-	-
DBA	10 to 90	-	-	-	-	-	-	-	-
VVA	10 to 90	-	-	-	-	-	-	-	-
LCA	10 to 90	-	-	-	-	-	-	-	-
PSA	10 to 90	-	-	-	-	-	-	-	-
UEA-I	10 to 90	-	-	-	-	-	-	-	-
LTA	10 to 90	-	-	-	-	-	-	-	-

BV = blood vessels, CRL = crown rump length, CT = connective tissue E = epithelium, PMC = peritubular muscle coat.

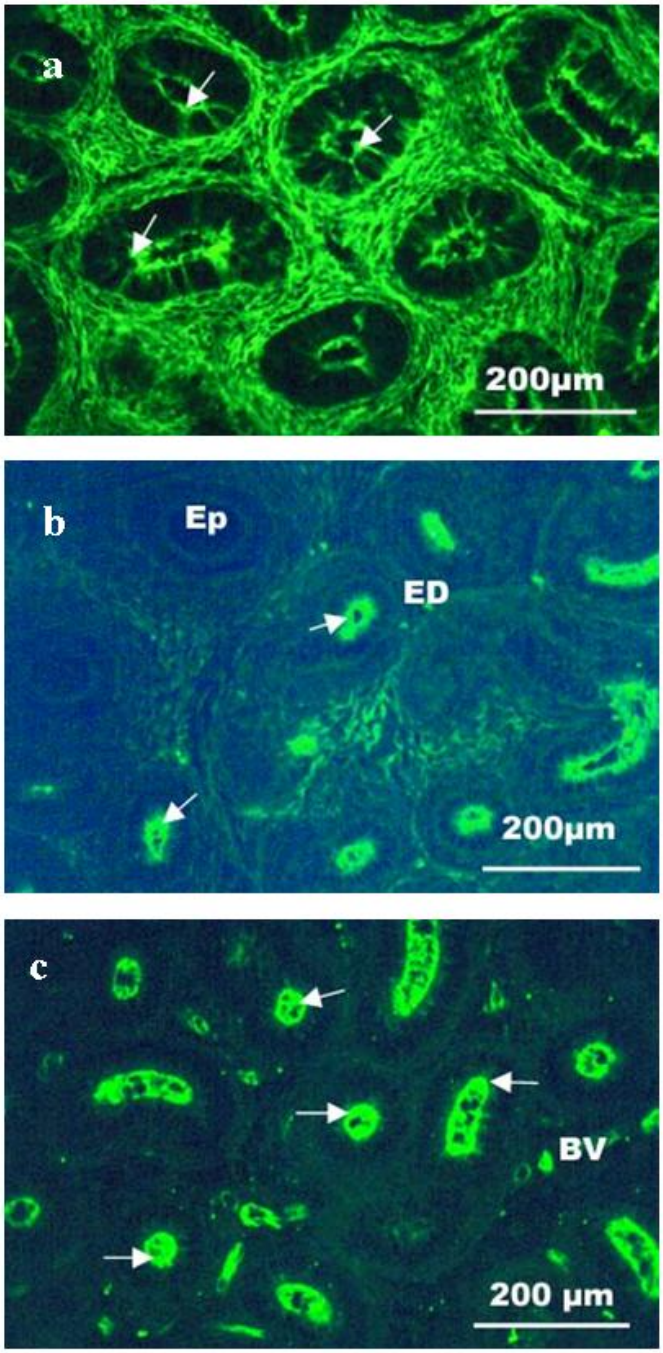


Fig. 8: Binding sites of FITC-lectins in foetal bovine efferent ductules and epididymis. WGA-binding sites in efferent ductules from 56 cm (CRL) long foetus (a). PNA-binding sites in efferent ductules from 30 cm (CRL) long foetus (b). GSA-I-binding sites in efferent ductules from 63 cm (CRL) long foetus (c). ED = efferent ductules, Ep = epididymal duct, BV = blood vessels.

The epithelium of the epididymal duct reacted negatively throughout its entire length, however the periductal cells and connective tissues expressed positive reaction. PNA-binding sites were also restricted to the apical surface of the epithelium of ED (Fig. 8b) from foetuses of CRL 10 cm (75 pcd, 3rd gestational month) and upwards. On the other hand, epididymal epithelium expressed no PNA-staining (Fig. 8b). GSA-I-binding sites exhibited a reaction pattern similar to that of PNA; moreover the blood vessels show GSA-I-staining (Fig. 8c).

4.1.3 Immunohistochemical studies of bovine prenatal epididymis (Table 7)

4.1.3.1 Immunolocalization of S-100

At the foetal age of 75 to 80 pcd (CRL, 10 to 13 cm), the epithelium of the efferent ductules expresses moderate (CRL, 10 cm) to strong (CRL, 13 cm) S-100-immunoreactivity. The epididymal epithelium exhibits no immunoreaction. The immunoreactivity expressed by the vascular endothelium varies from moderate to strong. At the foetal age of 95 to 110 pcd (CRL, 18 to 24 cm), most cells of the epithelium lining the efferent ductules exhibit a moderate reactivity, whereas some scattered ciliated cells express a strong immunoreaction. The degenerating mesonephric tubules show a reaction pattern similar to that exhibited by the epithelium lining the efferent ductules. The vascular endothelium shows a strong positive reactivity for S-100. Many fibroblasts are also positive for S-100. The epithelium of the epididymal duct in the head region manifests weak to marked S-100-immunoreaction especially in the apical cytoplasm, whereas the epithelium in the body and tail regions appears negative. At the foetal age of 130 to 140 pcd (CRL, 30 to 36 cm), the epithelium of the efferent ductules exhibits a similar pattern of reactivity as in the previous stages, but the number of strongly positive ciliated cells has increased among the majority of the moderately reactive nonciliated cells.The epithelium of the epididymal duct in the head region expresses moderately reactive stereocilia and cytoplasm. The epithelium lining the ductus epididymidis in the body and proximal tail region exhibits a weak to moderate apical surface and supranuclear cytoplasm. Also the infranuclear cytoplasm of some cells is positive. The epithelium of the epididymal duct in the most distal region of the tail expresses stronger immunoreactivity for S-100 than the more proximal tail region.

At the foetal age of 185 pcd (CRL, 56 cm) and upwards, the efferent ductules exhibit a pattern of S-100-immunoreaction similar to that expressed by the previous stages, but the strongly positive ciliated cells become more numerous than before (Fig. 9a, 9b). The more frequent nonciliated cells present a moderate reactivity including cytoplasm and nuclei. The epithelium of the epididymal duct in the head region expresses moderate to strong immunoreactivity for S-100 particularly of the stereocilia, apical surface and cytoplasm as well as most of the nuclei (Fig. 9a).The epithelium lining of the body region exhibits moderate immunoreaction. This includes positive granules in the apical cytoplasm of most cells as well as positive nuclei and infranuclear cytoplasmic granules of some cells. In the tail region, the epithelium shows two different patterns of reactivity. The epithelium lining of the proximal cauda expresses a signal similar to that of the body region, whereas the most distal portion of the duct expresses

strongly reactive cytoplasm and nuclei. This may correlate to the notion that this portion begins to differentiate in the neonates earlier than the other regions of the epididymal duct.

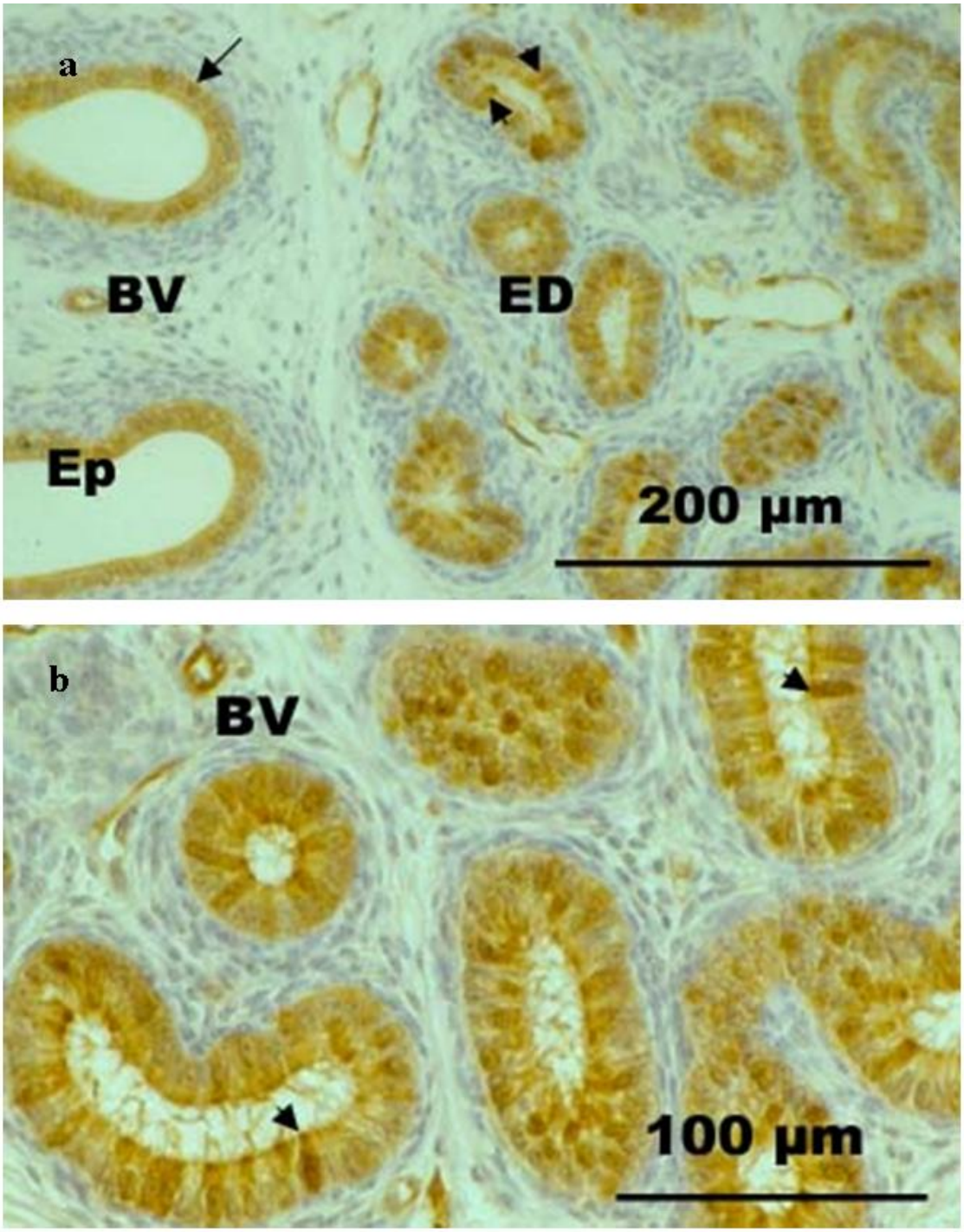


Fig. 9: S-100-immunolocalization in (a) efferent ductules and epididymal duct from a bovine foetus of 56 cm CRL and in (b) efferent ductules from a foetus of 63 cm CRL; the arrow heads point to ciliated cells in efferent ductules and the arrows point to positive nuclei of the epididymal epithelial cells. BV = blood vessels, ED = efferent ductules, Ep = epididymal duct.

4.1.3.2 Immunolocalization of aFGF (FGF-1)

At the foetal age of 75 to 110 pcd (CRL, 10 to 24 cm), the apical cytoplasm of the epithelium lining both the efferent ductules and epididymal duct in the head region expresses strongly aFGF-immunoreactive granules. The epithelium of the ductus epididymidis in the body and

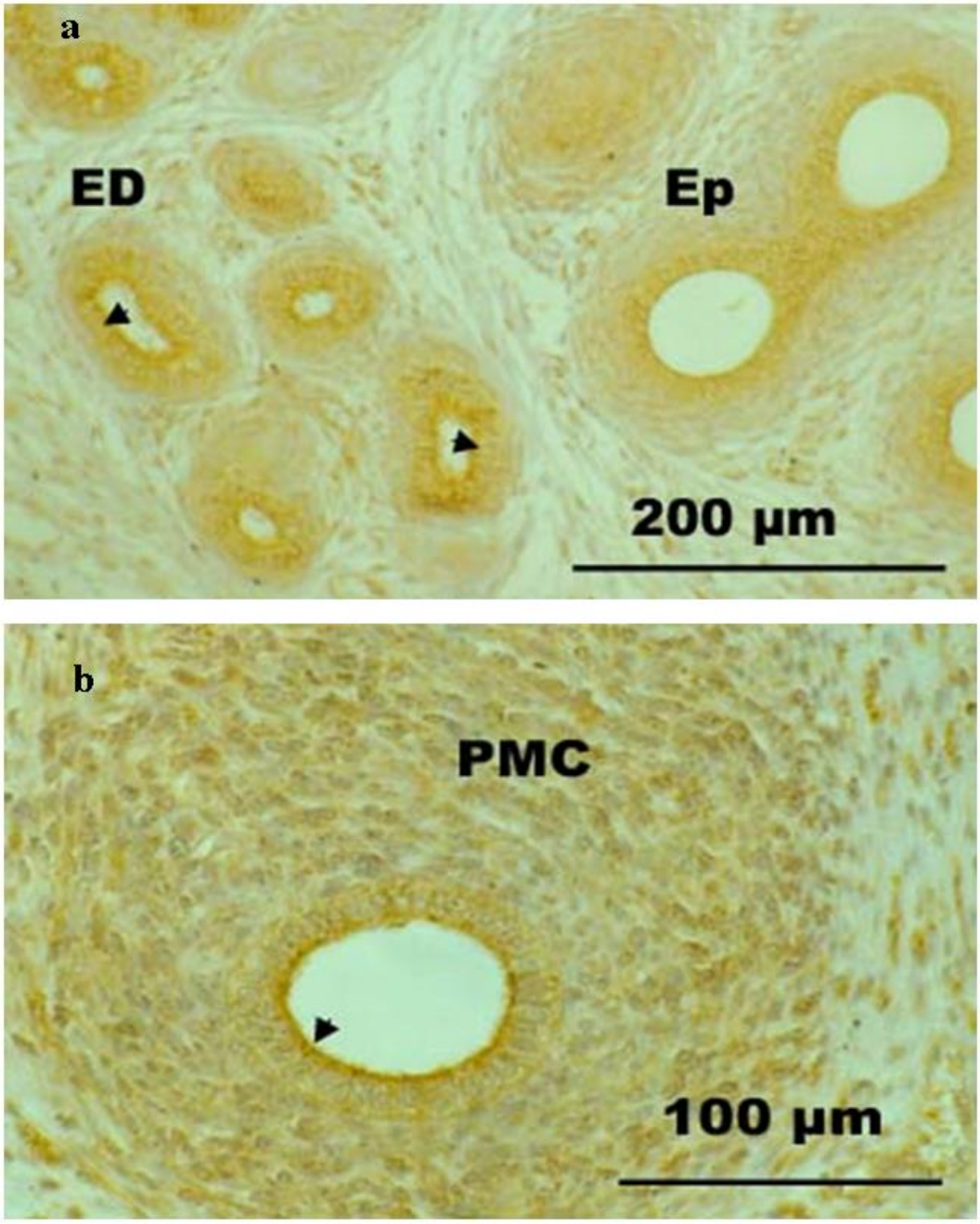


Fig. 10: FGF-1-immunolocalization in (a) efferent ductules and caput epididymidis from a foetus of 56 cm CRL and in (b) cauda epididymidis from 30 cm (CRL) long foetus; arrow heads point to the markedly positive apical cytoplasm both in efferent ductules and epididymis. ED = efferent ductules, Ep = epididymal duct, PMC = peritubular muscle coat.

tail region exhibits a signal similar to that of the head region but the number of positive granules is apparently fewer.

At the foetal age of 130 to 140 pcd (CRL, 30 to 36 cm), the apical cytoplasm of the epithelium of the efferent ductules and the epididymal duct in the head region displays homogenous positive reaction as well as strongly reactive granules scattered throughout this cytoplasmic area. A few FGF-1-positive granules are found in the infranuclear cytoplasm and in the peritubular cells. The epithelium of the body region shows a reaction pattern similar to that expressed by the head region. In the cauda epididymidis the epithelium shows numerous positive granules in the apical cytoplasm (Fig. 10b). The peritubular cells present some FGF-1-positive granules. Also many fibroblasts and the vascular endothelium exhibit positive FGF-1-immunoreaction. At the foetal age of 185 pcd (CRL, 56 cm) and upwards, the epithelium lining the efferent ductules shows a distinctly reactive apical cytoplasm with strongly positive granules (Fig. 10a). Also the epithelium of the epididymal duct shows strongly positive granules dispersed in a moderately positive homogenous apical zone of cytoplasm (Fig. 10a). Some positive granules are also found in the basal cytoplasm. A few FGF-1-positive granules are found in the peritubular smooth muscle cells.

4.1.3.3 Immunolocalization of bFGF (FGF-2)

At the foetal age of 75 to 80 pcd (CRL, 10 to 13 cm), the apical cytoplasm of the efferent ductules expresses moderate (CRL, 10 cm) to strong (CRL, 13 cm) positive granules in the apical cytoplasm. Similar granules are also found in the basal cytoplasm. Some cells possess a strongly positive apical plasma membrane. Also some nuclei exhibit moderate to strong FGF-2-immunoreactivity. The number of strongly positive nuclei increases with foetal age. The epithelium of the epididymal duct in the head region shows a weak to moderate FGF-2-immunostaining. The immunoreactivity in the epithelium lining of the ductus epididymidis in the body and tail region is more intense than that in the head region. Furthermore, the number of FGF-2-immunopositive nuclei is highest in the tail region followed by the body region. Though the peritubular cells show no immunoreaction, the extracellular matrix displays a weak to moderate reaction (Fig. 11a) which disappears with advance of the foetal age. The vascular endothelium presents a moderate FGF-2-immunoreactivity. At the foetal age of 110 pcd (CRL, 24 cm), the immunoreaction for FGF-2 is similar to that of 13 cm CRL. In foetuses with a CRL of 30 to 36 cm (130 to 140 pcd), the pattern of FGF-2-immunoreactivity is similar to the previous stages, but the intensity of the reaction has increased (Fig. 11a). At the foetal age 185 pcd (CRL, 56 cm) and upwards, the distribution pattern of FGF-2-immunoreaction in

the epithelium lining the efferent ductules is similar to the former stages, but the signal is more intense both in cytoplasm and nuclei. Furthermore, the number of the positive nuclei has considerably increased. Many cells lining a cross section of an efferent ductule show FGF-2-positive nuclei (Fig. 11b).

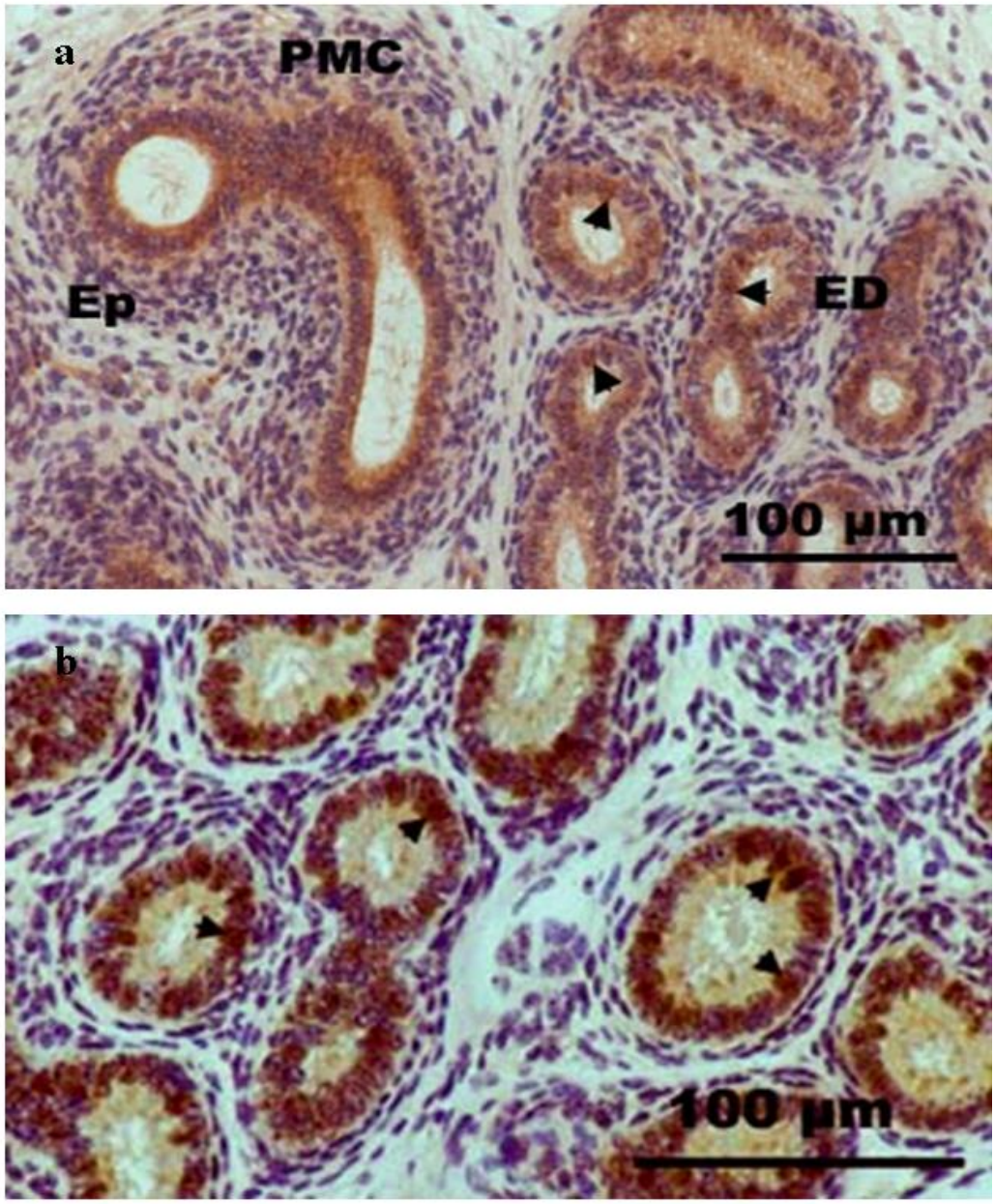


Fig. 11: FGF-2-immunolocalization in (a) efferent ductules and caput epididymidis from 36 cm (CRL) long foetus and in (b) efferent ductules from 63 cm (CRL) long foetus; arrow heads point to strongly positive nuclei of ciliated cells of efferent ductules. ED = efferent ductules, Ep = epididymal duct, PMC = peritubular muscle coat.

The epithelium of the epididymal duct in the head region presents a weak immunoreactivity for FGF-2 and no immunopositive nuclei are found. The epithelium of the epididymal duct in

the body and tail region expresses a weak to moderate cytoplasmic immunoreactivity. Furthermore, many nuclei exhibit a moderate to strong signal of immunoreactivity, which increases with the foetal age. The numbers of the immunopositive nuclei are higher in the cauda than in the corpus region and increase with foetal age. Some nuclei of the peritubular smooth muscle cells also exhibit a marked immunoreactivity for FGF-2.

4.1.3.4 Immunolocalization of Angiotensin Converting Enzyme (ACE)

At the foetal age of 75 to 80 pcd (CRL, 10 to 13 cm), the efferent ductular epithelium shows a moderate ACE-immunoreactivity in the apical cell membrane, whereas the reactivity of the cytoplasm is weak (Fig. 12a). In the epididymal duct, both the cytoplasm and apical cell membrane express a weak to moderate reaction (Fig. 12a). The vascular endothelium exhibits a moderate to strong reactivity. At the foetal age of 95 to 110 pcd (CRL, 18 to 24 cm), a moderate to strong reactivity is expressed in the apical plasma membrane and cytoplasm of the efferent ductules and the epididymal duct in the head region. Strongly ACE-positive, fine granules are found in the cytoplasm of the epithelial cells in these regions. A similar reaction is found in the degenerating mesonephric tubules in the area opposite to the body region of the epididymis. The epididymal epithelium in the body and tail region expresses a weak to moderate reaction both on the apical surface and in the cytoplasm. The peritubular mesenchymal cells as well as many of the interstitial cells present a weak to moderate immunoreactivity for ACE.

At the foetal age of 130 to 140 pcd (CRL, 30 to 36 cm), the apical surface of the epithelium lining the efferent ductules exhibits a strong positive reaction, whereas the apical cytoplasm shows a weak to moderate reaction. The epididymal epithelium in the head region expresses a moderate reaction in the apical cytoplasm and surface. Furthermore, some cells possess a moderate reaction in their basal cytoplasmic area. The reaction in the epithelium lining the duct in the corpus and cauda region is similar to that in the caput region. The vascular endothelium possesses a strong ACE-immunoreactivity.

At the foetal age 185 pcd (CRL, 56 cm) and upwards, the epithelium of the efferent ductules (Fig. 12b) presents a strongly immunoreactive apical surface, as well as a weak to moderate reactive apical cytoplasm containing ACE-immunopositive granules.The epithelium of the epididymal duct in the head region manifests a moderate to strong positive reactivity on the apical surface and positive granules in the apical as well as in the basal cytoplasm. The apical surface and cytoplasm in the body and tail region express a moderate reactivity. The apical cytoplasm of most cells presents ACE-positive granules, whereas some cells possess similar

granules also in their basal cytoplasm. The vascular endothelium exhibits a strong ACE-immunoreactivity.

4.1.3.5 Immunolocalization of Galactosyltransferase

At the foetal age of 75 to 110 pcd (CRL, 10 to 24 cm), the epithelium of the efferent ductules and the epididymal duct shows a moderately positive supranuclear cytoplasm. The intensity of GT-immunoreaction increases with foetal age.

At a foetal age of 130 to 140 pcd (CRL, 30 to 36 cm), the pattern of the immunoreactivity is similar to that in the previous stages, but the signal of the reaction is somewhat stronger. Both of the cilia (efferent ductules) and stereocilia (epididymal duct) showed a positive immunoreaction.

At the foetal age of 185 pcd (CRL, 56 cm) and upwards, the efferent ductules still expressed moderate positive immunoreaction in the apical cytoplasm of their epithelium. The epithelium lining the different regions of the epididymis also showed a moderate reaction in the apical cytoplasm and stereocilia (Fig. 12c). The peritubular smooth muscle cells, particularly those of the innermost layers expressed a moderate immunoreactivity for GT.

4.1.3.6 Immunolocalization of α-Smooth Muscle Actin (α-SMA)

At the foetal age of 75 pcd (CRL, 10 cm), some periductular cells of some efferent ductules express a moderate immunoreaction for α-SMA. This reaction first appears in the innermost layer of the periductular cells. The peritubular cells surrounding the epididymal duct are negative at this time. The smooth muscle cells of blood vessels are strongly reactive especially in the large vessels.

At the foetal age of 80 pcd (CRL, 13 cm), the reactivity for α-SMA is similar to that in the former stage (CRL, 10 cm) but most of the efferent ductules exhibit positively reactive periductular cells. The peritubular cells surrounding the epididymal duct are still negative for α-SMA.

At the foetal age of 95-110 pcd (CRL, 18-24 cm), the periductular cells express a moderate reaction in a concentric pattern surrounding the efferent ductules. The reaction becomes more diffuse even in the outer layers. The peritubular cells surrounding the epididymal duct exhibit a positive reaction. The intensity of the reaction was moderate to strong in the innermost layers, whereas the outer layer reacted in a weaker manner.

At the foetal age of 130 to 140 pcd (CRL, 30-36 cm), the periductular cells exhibit a moderate and strong reactivity in the outer and inner layers respectively (Fig. 12d). Similarly, in the

epididymal duct, the innermost layers of the peritubular cells are strongly reactive, while the outermost ones reacted only moderately. The vascular smooth muscle cells express a moderate to strong positive immunoreaction for α-SMA.

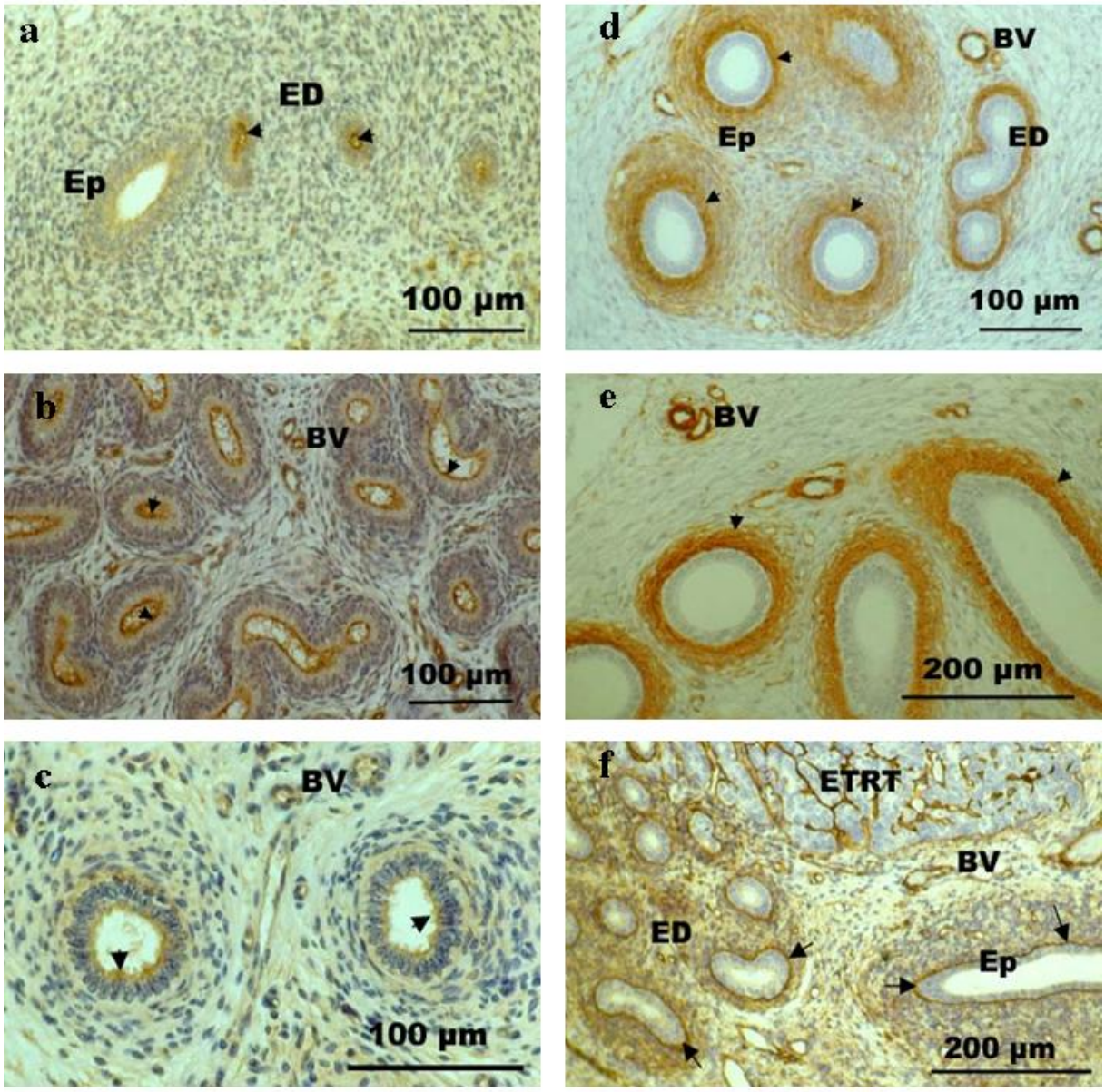


Fig. 12: Immunolocalization of ACE in efferent ductules and caput epididymidis from 13 cm (a) long (CRL) bovine foetus and in efferent ductules from 63 cm (b) long (CRL) foetus; arrow heads point to markedly positive apical surface of efferent ductules. Immunolocalization of GT in stereocilia and apical surface (arrow heads) of the epithelium of cauda epididymidis (c) from a foetus with 90 cm CRL. Immunolocalization of α-SMA in peritubular cells of efferent ductules and caput epididymidis from 30 cm (d) long (CRL) foetus and in cauda epididymidis (e) from 56 cm long (CRL); arrow heads point to the more intense reactivity in the innermost layers of peritubular cells. Immunolocalization of laminin (f) in extratesticular rete testis, efferent ductules and caput epididymidis from 13 cm long (CRL) foetus; arrows point to markedly positive basal lamina. BV = blood vessels; ED = efferent ductules, Ep = epididymis, ETRT = extratesticular rete testis.

At the foetal age of 185 pcd (CRL, 56 cm) and upwards, the periductular smooth muscle cells surrounding the efferent ductules exhibit a strong positive reaction. The peritubular smooth

muscle cells surrounding the epididymal duct express moderate and strong positive reactivity in the outer and inner layers respectively (Fig. 12e). The vascular smooth muscle cells manifest a strong reactivity for α-SMA.

4.1.3.7 Immunolocalization of Laminin

The reactivity for laminin appears as early as in the 75 to 80 days old foetus (CRL, 10 to 13 cm); however the overall reaction is moderate to strong in different elements. The basal lamina both of efferent ductules and epididymal epithelium exhibits a distinctly positive reaction (Fig. 12f). The peritubular mesenchymal cells also show a positive reactivity. The vascular endothelial basal lamina is also positive. The intensity of immunoreactivity increases with foetal age.

4.1.3.8 Immunolocalization of Connexin-43

No immunoreaction for connexin-43 could be localized in the prenatal efferent ductules or epididymal duct, neither in the epithelium nor in the peritubular smooth muscle cells or in the interstitium.

Table 7: Immunolocalization of some functional proteins in foetal efferent ductules and epididymis.

Protein	CRL (cm)	Efferent ductules (ED)					Epididymal duct			
		CC	NC	PMC	BL	BV	E	PMC	BL	BV
S-100	10-13	+	+	-	-	+/++	-	-	-	+/++
	18-24	+++	+	-	-	+++	±/+	-	-	+++
	30-36	+++	+	-	-	+++	+/++	-	-	+++
	56-90	+++	+	-	-	+++	+/++	-	-	+++
FGF-1	10-13	++	++	±	-	±	++	±	-	±
	18-24	++	++	±	-	±	++	±	-	±
	30-36	+++	+++	+	-	+	+++	+	-	+
	56-90	+++	+++	+	-	+	+++	+	-	+

Protein	CRL (cm)	Efferent ductules (ED)					Epididymal duct			
		CC	NC	PMC	BL	BV	E	PMC	BL	BV
FGF-2	10-13	++	±	-	-	+	±/+	-	-	±
	18-24	++	±	-	-	+	±/+	-	-	±
	30-36	+++	±	-	-	+	+/++	-	-	±
	56-90	+++	±	-	-	+	++	-	-	±
ACE	10-13	+	+	±	-	++	±/+	±	-	++
	18-24	+/++	+/++	±	-	++	+	±	-	++
	30-36	+++	+++	±	-	+++	++	±	-	+++
	56-90	+++	+++	±	-	+++	++	±	-	+++
GT	10-13	±/+	±/+	±	-	±	±/+	±	-	±
	18-24	±/+	±/+	±	-	±	+	±	-	±
	30-36	+	+	±	-	±	+	±	-	±
	56-90	+	+	±	-	±	+	±	-	±
α-SMA	10-13	-	-	+/++	-	++	-	-	-	++
	18-24	-	-	++	-	++	-	+/++	-	++
	30-36	-	-	+++	-	+++	-	++	-	+++
	56-90	-	-	+++	-	+++	-	+++	-	+++
Laminin	10-13	-	-	+/++	++	++	-	+/++	++	++
	18-24	-	-	++	++	+++	-	++	++	+++
	30-36	-	-	+++	+++	+++	-	+++	+++	+++
	56-90	-	-	+++	+++	+++	-	+++	+++	+++
Connexin 43	10-13	-	-	-	-	-	-	-	-	-
	18-24	-	-	-	-	-	-	-	-	-
	30-36	-	-	-	-	-	-	-	-	-
	56-90	-	-	-	-	-	-	-	-	-

BL = basal lamina and BV = blood vessels both of ED and epididymal duct. CC = ciliated cells of ED, CRL = crown rump length, E = epididymal epithelium, NC = nonciliated cells of ED, PMC = peritubular muscle coat, - = negative, ± = weak, + = moderate positive, ++ = distinct positive, +++ = strong positive.

4.2 Adult bovine epididymis

4.2.1 Microscopic anatomy of the adult bovine epididymis

4.2.1.1 Efferent ductules

The bovine efferent ductules (ED) are arranged in lobules with indistinct boundaries of connective tissue. ED are lined by ciliated simple columnar epithelium (Fig.13). The epithelium consists of ciliated and nonciliated columnar cells. Ciliated cells are distinguished not only by the cilia but also by the more apical position of their nuclei within the epithelium. They possess ovoid or pear-shaped heterochromatic dark nuclei. Their luminal surface is provided by kinocilia. Both of intraepithelial lymphocytes and macrophages are found scattered throughout the epithelium and are characterized by heterochromatic dense nuclei.

The nonciliated cells are classified according to their cytological features into three categories: the first type contains neither granules nor vacuoles (Fig.13a), the second type possesses granules (Fig.13b), whereas the third type is characterized by vacuoles (Fig.13c). The luminal surface of all the three cell types is provided with microvilli, apical protrusions, or both of them. These apical protrusions may contain granules or vacuoles (Fig.13b). They are also found separated in the lumen, along with granules and vacuoles. The nuclei of all three cell types are oval, moderately basophilic and located in the basal third of the cell. The specific granules of the second type of nonciliated cells stain with eosin and PAS (Fig.13b) but are negative with Alcian blue. The vacuoles of the third type of nonciliated cells do not stain with eosin, PAS (Fig.13c) or Alcian blue. The vacuoles exist in the supranuclear half of the cell, but unlike the granules they were never seen in the infranuclear area.

In addition to the three typical nonciliated cell types, another type of cells is occasionally encountered. The occurrence of this cell type is restricted to the middle area of the efferent ductules. These cells contained both granules and vacuoles. However, unlike those of the second type of nonciliated cells, the granules are smaller, less dense and confined to the intermediate supranuclear region. The vacuoles occupy the remaining apical cytoplasm. Similar to the other cell types, the luminal surface was modified to form a microvillous border or apical protrusions. The distribution of the different types of nonciliated cells varied in the various regions of ED. The latter are divided into proximal, middle and distal areas. The first type is found throughout the entire length of the ED. The second type predominates in the

proximal area whereas the third type predominates in the distal area of the ED. However, simultaneous occurrence both of the second and the third types in the middle area could be encountered. This may be due to the overlap of segments.

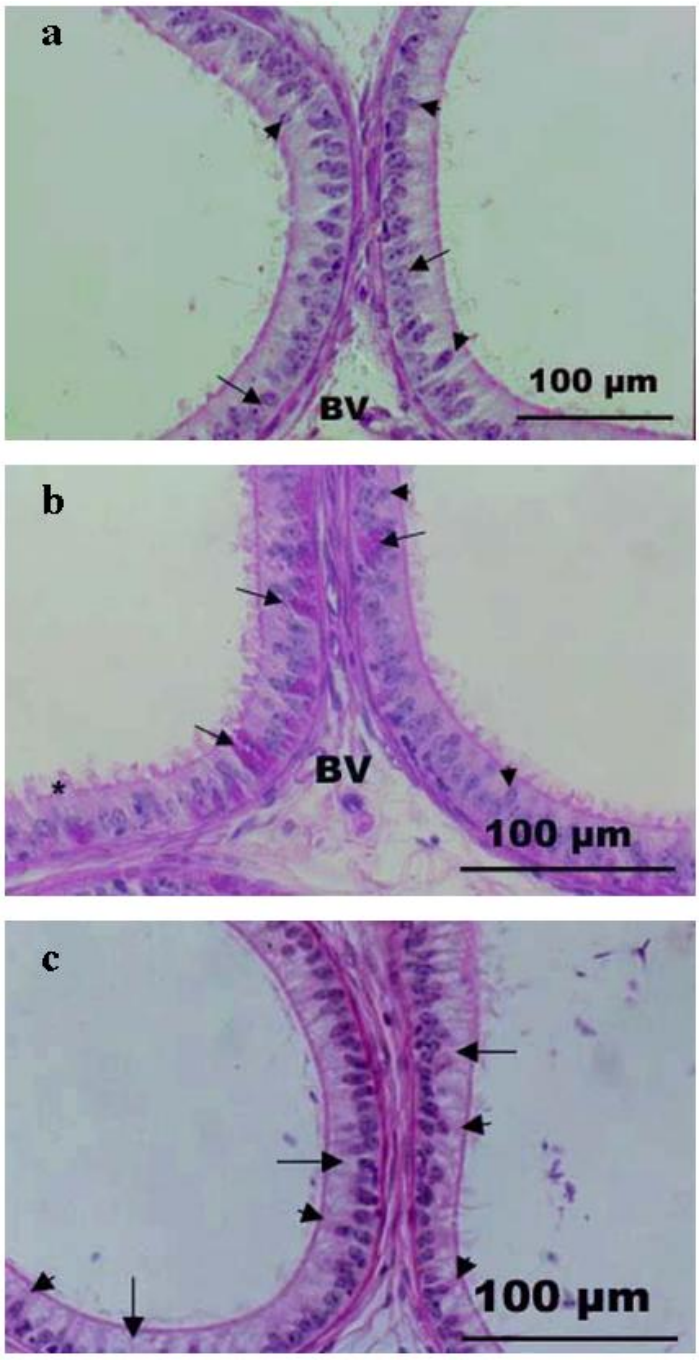


Fig.13: PAS-stained efferent ductules showed nonciliated type I cells (a) , nonciliated type II cells (b) and nonciliated type III cells (c). Arrows point to nonciliated cells, arrow heads point to ciliated cells, asterisk points to an apical protrusion (b), BV = blood vessels.

4.2.1.2 Epididymal duct

The epididymal duct is lined by a pseudostratified epithelium. It is characterized by two main cell types that can be seen along the entire length of the duct. They are the principal cells (PC) and the basal cells (BC) resting on a strongly PAS-positive basal membrane (Fig. 14). The apical borders of the PC are equipped with stereocilia, which exhibited a weak to moderate PAS-reaction. In addition to both cell types, apical mitochondria-rich cells (AMRC) were frequently found in certain segments of the duct. Intraepithelial lymphocytes (IEL) and macrophages (IEM) are found throughout the entire length of the epididymal duct. The IEL are present at different levels of the epithelium. They possess dark round nuclei, which

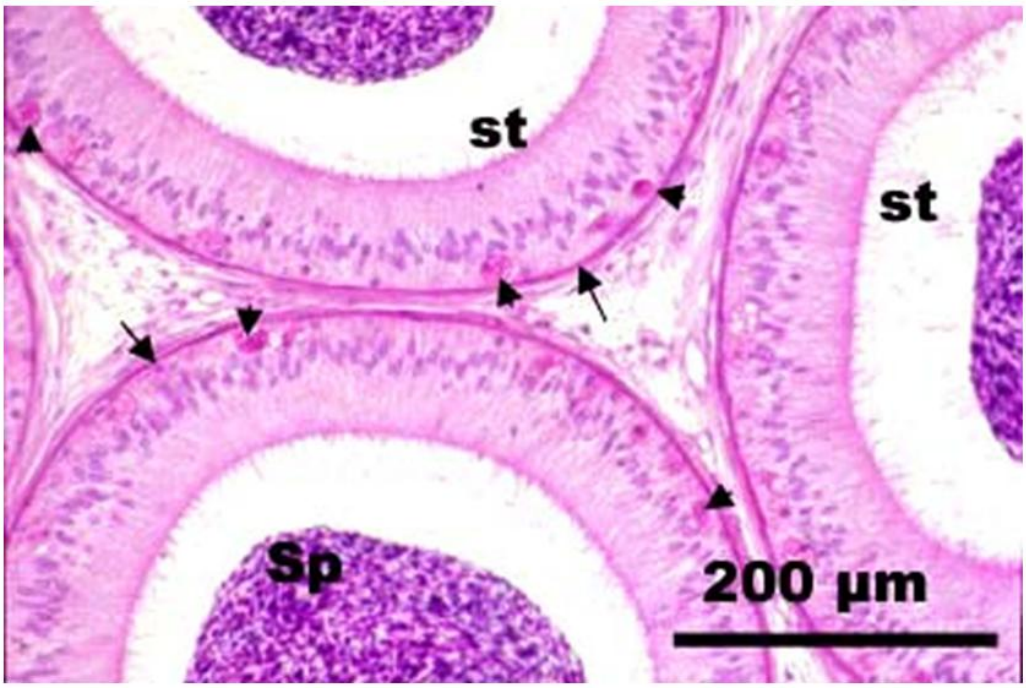


Fig. 14: PAS-stained adult bovine epididymal segment III, arrow heads point to strongly positive intraepithelial macrophages, mostly next to strongly positive basal membrane (arrows). Sp = spermatozoa, st = stereocilia.

are surrounded by a narrow cytoplasmic rim. Many cells are found in the basal area of the epithelium showing strongly PAS-positive, diastase-resistant granules (Fig. 14). These cells are seen along the entire length of the epididymal duct and are likely IEM. In H&E-stained sections they appear as large cells with dark eccentric nuclei and surrounded by a conspicuous cytoplasm, which sometimes contained eosinophilic structures or many large vacuoles (Fig. 18e). The latter gave them the foamy appearance (Erkmann, 1971). Similar cells are seen in the lumina of some tubules. The epididymal duct is surrounded by a thin lamina propria of fine collagen fibres and fibroblasts as well as a peritubular muscle coat (PMC).

The interstitium contains loose connective tissue in which the epididymal blood and lymph vessels and nerve fibres are distributed. Many different cell types are found in the interstitum. These cells are mainly fibroblasts as well as mast cells, macrophages, lymphocytes and plasma cells. Mast cells are positively stained with Toludin blue (Fig. 15) and Alcian blue (Fig. 16).

Table 8: Morphometric values of the different segments of the bovine epididymal duct.

S	Epithelial height	Stereocilia	Nuclear size	PMC	Luminal diameter
I	105.39 ± 6.81	21.99 ± 0.79	17.32 ± 0.99	26.01 ± 1.76	260.80 ± 32.47
II	77.01 ± 4.19	20.19 ± 0.53	11.74 ± 1.11	24.99 ± 2.64	272.80 ± 26.47
III	75.99 ± 3.14	19.60 ± 0.70	22.72 ± 0.68	25.80 ± 2.42	260.60 ± 22.17
IV	81.00 ± 2.64	17.04 ± 0.88	23.52 ± 0.97	27.80 ± 1.66	227.40 ± 08.35
V	92.79 ± 4.59	16.60 ± 0.81	25.92 ± 1.24	30.99 ± 1.42	220.60 ± 12.12
VI	53.60 ± 4.22	07.20 ± 0.47	21.48 ± 1.13	55.60 ± 9.05	429.20 ± 41.72

PMC = peritubular muscle coat, S = epididymal segment.

4.2.1.2.1 Segment I

A cross section of the duct in this region reveals an irregular contour varied from triangular to stellate-shaped and the apical border of the epithelium is festooned (Fig. 17a). This may be due to folding of the epithelium, since the epithelium presents tops (156 µm) and valleys (72 µm) and the mean epithelium height was 105.39 ± 6.81 µm. The nuclei of the PC are elongated oval and mostly located next to the basal membrane of the epithelium, however some nuclei can be seen at different levels of the epithelium and sometimes reach almost the apical epithelial plasma membrane.

The mean value of the nuclear size was 17.32 ± 0.99 µm. The nuclei are vesicular and possess one or more nucleoli. Some nuclei particularly those near the apical plasmalemma exhibit mitotic activity. The apical cell membrane of the PC is provided with long stereocilia (21.99 ± 0.79 µm). The latter exhibit a strong positive reaction with Alcian blue (Fig. 16). Faintly Alcian blue-stained cytoplasmic granules are also seen in the PC of this segment. Many deep epithelial crypts are frequently found and are characteristic for this segment and when crossly cut, they appear like glandular acini (Fig. 17a). The free borders of the cells lining these crypts are also equipped with stereocilia. The apical cytoplasm of many PC presents vacuoles,

which sometimes contain pale eosinophilic, homogenous material (Fig. 18a). The nuclei of the BC are located in close contact with the basal membrane of the epithelium. They are often

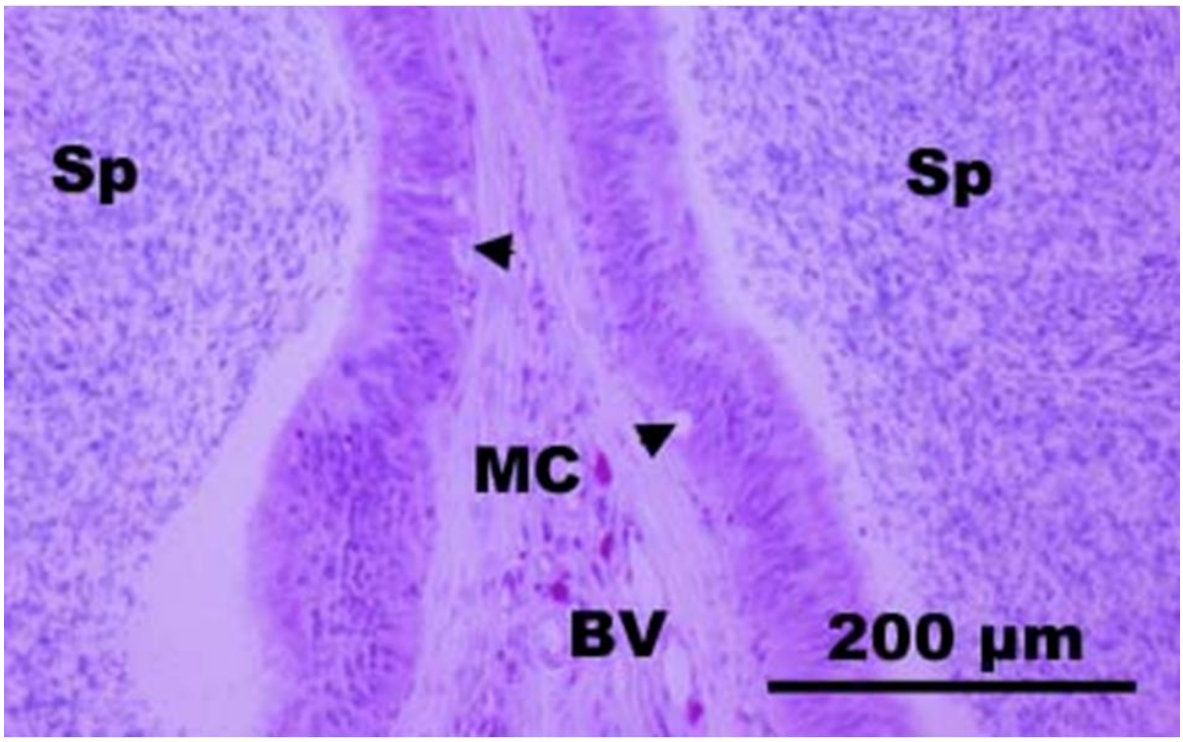


Fig. 15: Toluidin blue-stained adult bovine epididymal segment VI showing positive mast cells in the interstitium. BV = blood vessels, Sp = spermatozoa, MC = mast cells. Arrow heads point to negative intraepithelial macrophages.

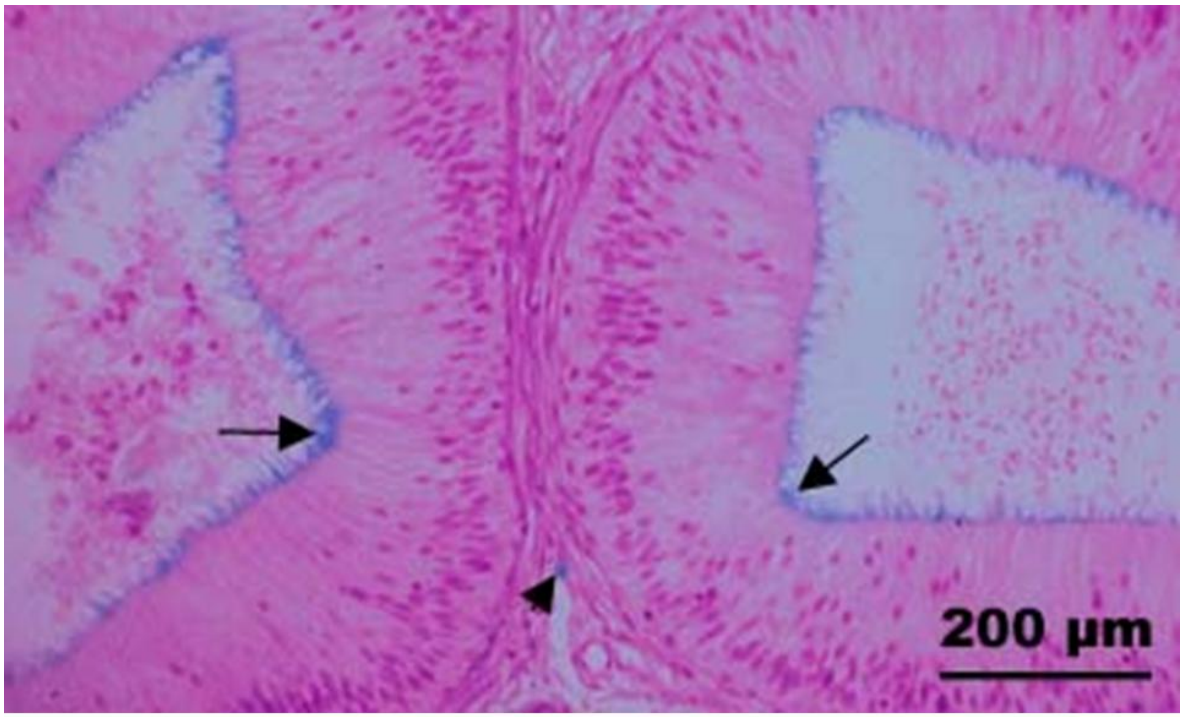


Fig. 16: Alcian blue-stained adult bovine epididymal segment I (a). Arrow head points to a positive mast cell in the interstitium. Arrows point to positive stereocilia.

pyramidal in shape. Some of them possess a flattened form. The mean luminal diameter reached 260.80 ± 32.47 µm and the lumina contain no or few numbers of spermatozoa as well as several spermatogenic cells. Furthermore, many eosinophilic homogenous granules are found. Some of them are attached to the apical surface of the epithelium. Intraepithelial leucocytes are often found near the basal membrane, but some of them are also seen at different levels of the epithelium. Their often round nuclei are surrounded by a narrow pale-stained cytoplasmic rim. These nuclei are smaller and darker than those of the principal and the basal cells. The PMC consists of about 5 concentrically arranged layers of smooth muscle cells (SMC). The mean value of the thickness of the PMC was 26.01 ± 1.76 µm.

4.2.1.2.2 Segment II

The lumina of this segment are regular oval or round and are surrounded by a uniform epithelium (Fig. 17b) without foldings as those found in segment I. The epithelium is lower than that of the first segment and the mean epithelial height was 77.01 ± 4.19 µm. The mean luminal diameter was 272.80 ± 26.47 µm. The lumina contain moderate amounts of spermatozoa, some intraluminal leucocytes and spermatogenic cells. Although the stereocilia are shorter (20.19 ± 0.53 µm) than those of the first segment, they are still of considerable length and may be stuck together. The nuclei of the PC are mostly short (11.74 ± 1.11 µm), ovoid or even round with two to three nucleoli (Fig. 18b).

As they are located in the basal area of the epithelium, the nuclei of the PC can not be easily differentiated from those of the BC. However, some of them are found near the apical end of the epithelium and some mitotic figures can be seen. The apical cytoplasm of the PC contains an abundance of vacuoles. Furthermore, apical protrusions are seen on some cells containing pale-stained vacuoles (Fig. 18b).

In the apical area of the epithelium a few AMRC are encountered. The numbers of intraepithelial leucocytes are similar to that of the first segment. The PMC is formed by about 5 layers of SMC and its mean thickness was 24.99 ± 2.64 µm.

4.2.1.2.3 Segment III

The lumen is regular oval or round and free from foldings. The mean luminal diameter was 260.60 ± 22.17 µm. The lumina are densely crowded with spermatozoa (Fig. 17c). The height of the epithelium is nearly similar to or slightly lower (75.99 ± 3.14 µm) than that of segment II. The apical cytoplasm of the PC shows abundance of pale-stained vacuoles. The stereocilia were similar to those of the second segment and their mean length was 19.60 ± 0.70 µm. The

nuclei of the PC are elongated oval and vesicular. They possess one or more nucleoli. The mean nuclear size was 22.72 ± 0.68 µm. They are located in the basal half of the cells leaving a considerable infranuclear cytoplasmic area and thus could be easily differentiated from those of the BC. Mitotic figures are frequently seen, particularly in the nuclei located in the upper half of the epithelium. They are more numerous than in the second segment. Also the BC are more frequent than in the former two segments. Their nuclei show variable size and shape and possess sometimes a nucleolus. AMRC and intraepithelial leucocytes are more frequent than in the previous two segments (Fig. 18c). The PMC is made up from about 5 layers of SMC and the mean value of its thickness was 25.80 ± 2.42 µm.

4.2.1.2.4 Segment IV

The lumina are in general oval or triangular (Fig. 17d). As a result of slight folding of the epithelium, some tubules present stellate-shaped lumina. The lumina of this segment are narrower than those of the previous segments. The mean luminal diameter was 227.40 ± 08.35 µm. The lumina possess many spermatozoa, however they are not as densely crowded as in segment III. The epithelium mostly possesses a uniform height, which is similar to that of the third segment or slightly higher (81 ± 2.64 µm). The apical borders of the PC are provided with stereocilia, which are somewhat shorter (17.04 ± 0.88 µm) than those of the third segment. The nuclei of the PC are elongated (23.52 ± 0.97 µm) with one or more nucleoli. They are darker than those of the head region. They are found at different levels of the epithelium. Most nuclei left a moderately wide infranuclear cytoplasmic zone so that they can be easily distinguishable from the nuclei of the BC. Mitotic figures are also seen in some nuclei found in the upper half of the epithelium. The number of the BC is higher than in the three segments of the head region. A small number of AMRC can be found in this segment. Intraepithelial leucocytes are frequently present in the basal area of the epithelium and sometimes also at higher levels. The PMC is formed by about 5 to 7 layers of SMC and attained a thickness of 27.80 ± 1.66 µm.

4.2.1.2.5 Segment V

The lumina of the epididymal duct in this region often present a stellate-shaped appearance (Fig. 17e). This results from the strongly folded epithelium, which constitutes tops (144 µm) and valleys (60 µm). The mean value of its height was 92.79 ± 4.59 µm. Thus the epithelium lining this segment is higher than that in both fourth and sixth segments. The luminal diameter is nearly similar to that of the fourth segment and its mean value was 220.60 ± 12.12 µm. The

lumina are filled with spermatozoa (Fig. 18e). The apical borders of the PC of this segment are provided with stereocilia, which are somewhat shorter (16.60 ± 0.81 µm) than those of the previous segments. The nuclei of the PC are elongated (25.92 ± 1.24 µm) and darker than those in the head region and possess one or more nucleoli. They are somewhat displaced

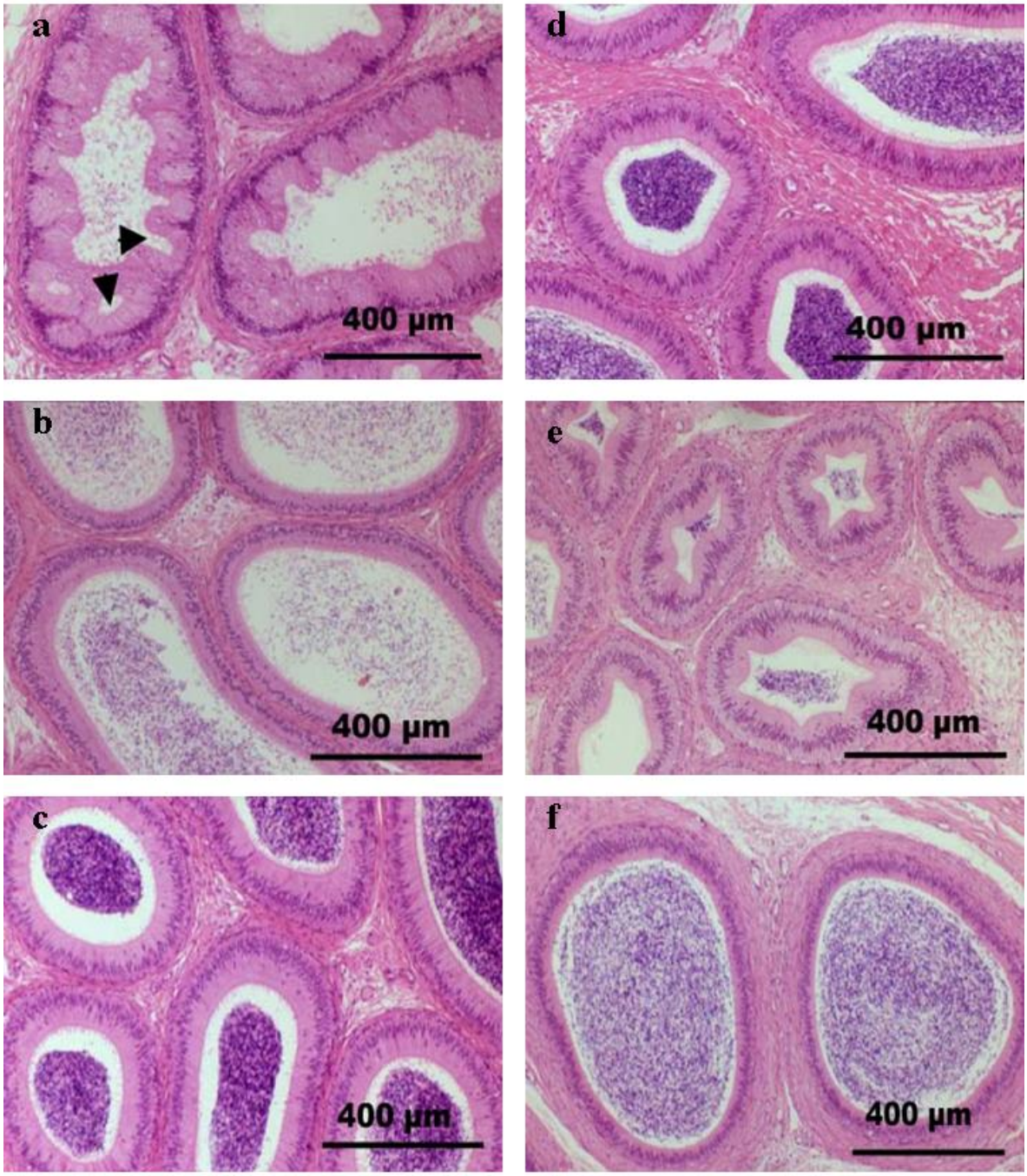


Fig. 17: H&E-stained sections of different segments of adult bovine epididymis. Letters a to f correspond to epididymal segments I to VI respectively. Arrow heads point to deep epithelial crypts which are characteristic for segment I (a).

towards the middle of the cells leaving a moderately wide infranuclear cytoplasmic zone. The nuclei of the BC are numerous and form a distinct cell layer (Fig. 18e). The AMRC are rarely found in this segment. The intraepithelial leucocytes (Fig. 18e) are similar to those in segment IV. About 5 to 7 layers of SMC constitute the PMC, whose mean thickness value was 30.99 ± 1.42 µm.

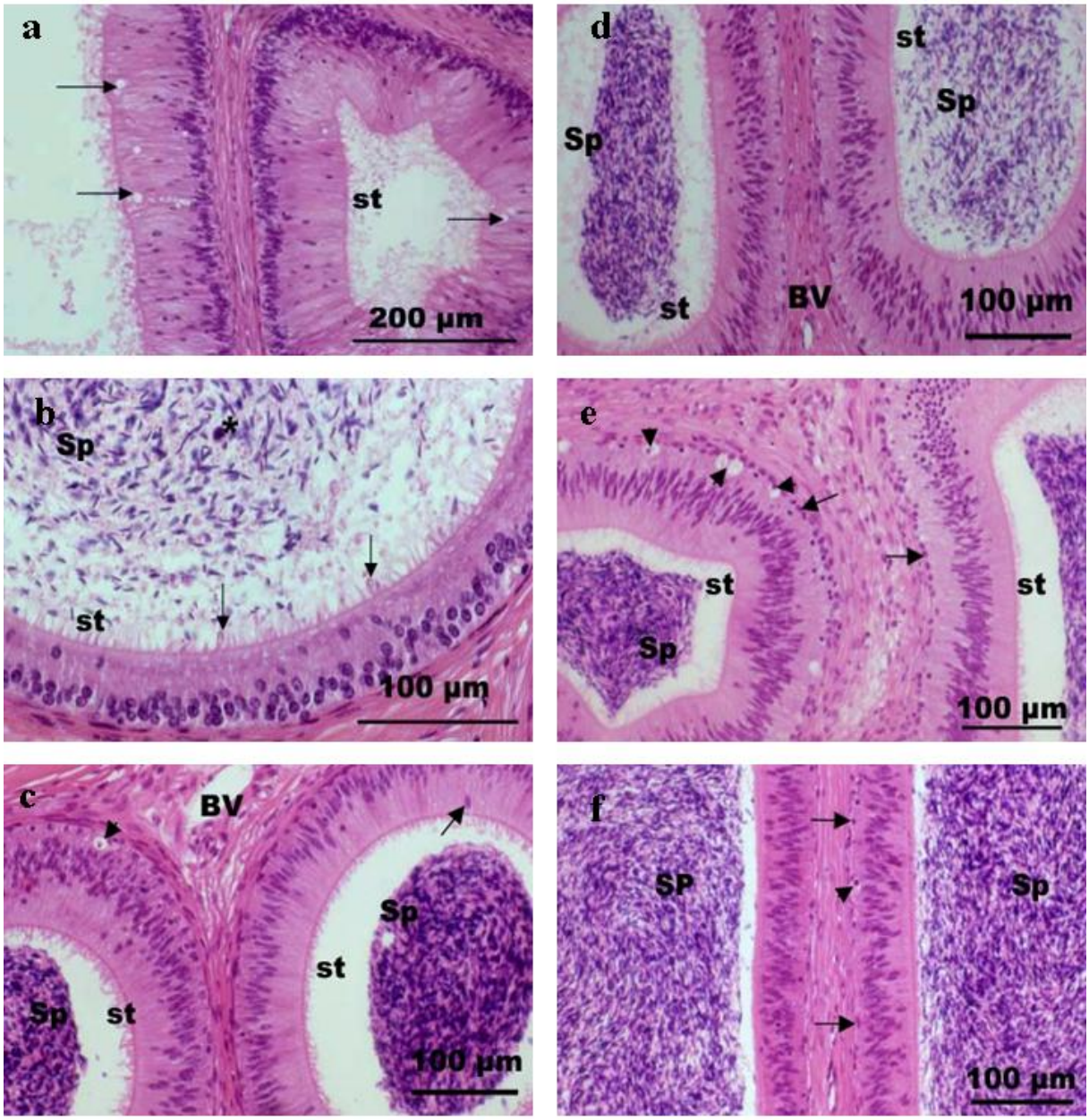


Fig. 18: H&E-stained adult bovine epididymal segments; a to f correspond to the segments I to VI respectively. Arrows point to cytoplasmic vacuoles (a), apical protrusions (b), apical cell (c) and basal cells (e and f); arrow heads point to IEM (c, e and f); asterisk = spermatogenic cells (b), BV = blood vessels, Sp =spermatozoa, st = stereocilia.

4.2.1.2.6 Segment VI

In comparison to other segments, the epithelium lining this segment of the epididymal duct is the lowest (Fig. 17f). The mean value of the epithelial height was 53.66 ± 4.22 µm. The height of the epithelium is uniform. The most proximal portion of this segment, however, possesses a slightly folded epithelium. The folding of the epithelium fades distalwards. The apical borders of the PC are equipped with dense and short (7.20 ± 0.47µm) stereocilia, which are the shortest when compared with those of other segments.

The nuclei of the PC are elongated (21.48 ± 1.13 µm) with one or more nucleoli. They are mainly located in the middle of the cells. Some of them are displaced apically leaving an infranuclear zone wider than the supranuclear one. When compared with those of other segments, the lumina are the widest (429.20 ± 4 1.72 µm) in the sixth segment. They are densely crowded with spermatozoa (Fig. 18f).

The BC are similar to those of the fifth segment, however their nuclei are often large, flat and loosely arranged. AMRC are never found in this segment. Intraepithelial leucocytes exhibit a similar morphology to those of the other segments; however they are more numerous. The PMC is formed by about 10 layers of concentrically arranged SMC. The mean value of its thickness was 55.60 ± 9.05 µm. It was the thickest layer of smooth muscle investment of the whole epididymal duct. The density of the capillary network in the interstitium is higher than in other epididymal segments.

4.2.2 Lectin binding sites of the adult bovine epididymis (Table 9, 10)

4.2.2.1 GSA-I-binding sites

The epithelium of the ED expresses alternatively negative ciliated and positive nonciliated cells (Fig. 19a). The latter presented positive apical cytoplasm and surface. Stereocilia of the PC, particularly in epididymal segments I and II express distinctly positive reactivity. Both BC and PC in the epithelium of the epididymal duct show a weak reaction with GSA-I. The Golgi zone (GZ) of the PC also shows a weak reaction. The vascular endothelium exhibits a moderate to distinct GSA-I binding throughout the entire length of epididymal duct and also in the ED. Luminal content, basal membrane, PMC and CT did not show any GSA-I binding sites.

4.2.2.2 PNA-binding sites

The epithelium of the ED presents no PNA-binding sites. Spermatozoa in their lumina exhibit a markedly positive reaction. A similar reaction has been seen in sperms in the lumina of the different segments of the epididymal duct (Fig. 19b). The epithelium of the epididymal duct reacts generally negative, however the PC of the segments IV and V reveal a weakly PNA-reactive GZ (Fig. 19b). Furthermore, stereocilia of PC of segment I, the vascular endothelium of the segments I and III and intraepithelial leucocytes display a weak binding of PNA.

4.2.2.3 ECA-binding sites

The ECA-binding sites in the epithelium of ED are represented by a positive apical surface and cytoplasm. In the epididymis, the cytoplasm of PC in segment I shows a moderate binding of ECA. Furthermore, the PC of the segments I and II manifest positive apical granules and stereocilia. GZ of both segments exhibits a weak ECA-binding. The luminal content exhibits a variable reactivity ranged from weak to distinct positive along the length of the duct. The intensity of reaction both of CT and the vascular endothelium ranges from weak to marked.

4.2.2.4 WGA-binding sites

In the epithelium of the ED negative ciliated cells alternate with positive, nonciliated cells. Binding sites are represented by a markedly positive apical surface and a positive basal membrane. The latter is also positive in the different segments of the epididymal duct. The BC express a distinct WGA-binding throughout the entire length of the duct. PC reveal also a marked reactivity along the length of the duct when incubated with FITC-WGA. The reaction is localized to stereocilia, GZ, and apical and basal cytoplasm (Fig. 19c). Furthermore, intraepithelial leucocytes also show WGA-binding. Vascular endothelium and CT react only weakly, whereas the PMC was negative. Intraluminal sperms show a distinct reaction (Fig. 19c).

4.2.2.5 Con A-binding sites

The epithelium of the ED consists of negative ciliated and positive nonciliated cells. In the epididymis the BC exhibit a distinctly positive Con A-binding throughout the entire length of the duct. The PC reveal a moderate reaction in segment I and a distinct reaction in the segments II through VI (Fig. 19d). The stereocilia of the PC of segment I show a moderate

reaction, whereas those of the segments II through VI display a remarkable binding of Con A. The luminal content is mostly negative along the whole length of the duct. Only the few spermatozoa of the first segment react positively. CT and vascular endothelium react weakly in segment I, whereas those of the other segments are negative.

4.2.2.6 LCA-binding sites

In the epithelium of the ED positive nonciliated cells alternate with negative ciliated cells. The nonciliated cells show moderately positive apical surface and protrusions, which may be released into the lumen. The PC of the first two segments of the epididymal head show moderately positive stereocilia and apical cytoplasm. A similar reaction is found in the fourth and fifth segments. The binding of LCA is more intense in the apical cytoplasm and stereocilia of the PC of the third segment (Fig. 20a). The GZ of the PC in the first segment manifests a weak reaction. In the fifth segment the PC exhibit a distinct LCA-binding in the GZ. The BC show a distinctly positive reaction throughout the entire length of the epididymal duct. The sixth segment shows a weakly positive epithelium with a moderate LCA-binding to stereocilia of the PC. The reactivity of the luminal content ranges from negative to weak. Intraepithelial leucocytes present a weak to distinct binding of LCA. The extratubular structures have no LCA-binding sites.

4.2.2.7 PSA-binding sites

There is no remarkable variation in the distribution of PSA-binding sites in the different regions of the epididymis. The epithelium of the ED expresses alternation between the positive nonciliated and the negative ciliated cells. The apical surfaces and stereocilia of the epididymal epithelium in the different segments react positively (Fig. 20b). The reaction is stronger in the first two segments. The GZ in the PC shows a moderate reaction in the third and the fifth segments and a weak reaction in the fourth segment. The epithelium of the sixth segment is entirely positive. The BC exhibit a moderately positive reaction throughout the entire length of the epididymal duct (Fig. 20b). Intraepithelial leucocytes express a distinct reactivity (Fig. 20b). The reactivity of the sperm cell mass in the lumina ranges from negative in the first two segments to weak in the other segments. Both of the basal membrane and the extratubular structures display no PSA-binding sites.

4.2.2.8 DBA-binding sites

No DBA-binding sites can be found in the ED. The marked DBA-binding sites show a great variation between the different segments of the epididymis. The reaction pattern in the first segment presents a remarkable variation between the neighbouring tubules. Whereas some tubules display no binding of DBA, other tubules show alternation between negative and positive groups of PC in their epithelium (Fig. 20c). Distribution of binding sites is confined to GZ, apical cytoplasm and stereocilia of the positive cells. The intensity of the reaction is distinct in GZ and moderate in the other two structures. The number of these positive cells ranges from sporadic cells in some tubules to most of the PC population in other tubules. The second segment possesses a generally DBA-negative epithelium. Only the apical cytoplasm and stereocilia showed a reasonably positive reaction. The GZ exhibited a weak reaction. The segments III to VI were completely negative. The PC of the fifth segment possess a GZ expressing a weak DBA-binding. Neither the sperm cells within the lumina nor extratubular structures present DBA-binding sites along the whole length of the duct.

4.2.2.9 HPA-binding sites

The apical surface and the GZ in some epithelial cells of the ED express a weak to moderate binding to HPA. Consequently, the pattern of reaction showed alternation between positive and negative cells. The distribution of HPA-binding sites reveals some variation between the different segments of the epididymis. Particularly in the first segment of the epididymal duct the HPA-binding pattern shows a great variation. Some tubules are completely negative, whereas other tubules display alternation of positive and negative groups of epithelial cells. The reaction is localized to the distinctly positive GZ and apical cytoplasm. The second segment manifests distinctly positive apical cytoplasm, stereocilia and GZ (Fig. 20d). The third segment expresses a moderately positive apical cytoplasm and stereocilia. In the fourth and fifth segments a moderately positive apical cytoplasm and GZ are seen. The sixth segment is negative. Intraluminal sperm cell mass and the extratubular structures show no HPA-binding sites along the entire length of the duct.

4.2.2.10 SBA-binding

Apical surface of efferent ductule epithelium reacts positively. In the epididymis the PC reveal weak and distinct binding of FITC-labelled SBA in the segments I and II respectively as well as a distinctly stained apical area. The other segments are negative. Vascular

endothelium and CT, only in segment III, are SBA-positive. The luminal content are negative in the different epididymal segments.

4.2.2.11 VVA-binding sites

Neither the ED nor the different segments of the epididymal duct show VVA-binding sites.

4.2.2.12 LTA-binding sites

LTA-binding sites could not be localized in the ED or in epididymal duct.

4.2.2.13 UEA-I-binding sites

Neither the ED nor the different epididymal segments display any UEA-I-binding sites.

Table 9: Distribution of binding sites of FICT-lectins in adult bovine efferent ductules.

Lectin	CC	NC	Lumen	BM	PMC	CT	VE
GSA-I	-	+	0	-	-	-	+
PNA	-	-	++	-	-	-	0
ECA	-	+	+	-	-	+	-
WGA	-	++	+	+	±	+	+
Con A	-	+	+	-	-	±	±
LCA	-	+	-	-	-	-	-
PSA	-	+	-	-	-	-	-
DBA	-	-	-	-	-	-	-
HPA	-	±	-	-	-	-	-
SBA	-	+	-	-	-	-	-
VVA	-	-	-	-	-	-	-
LTA	-	-	-	-	-	-	-
UEA-I	-	-	-	-	-	-	-

BM = basal membrane, CC = ciliated cell, CT = connective tissue, NC = nonciliated cell, PMC = peritubular muscle coat, VE = vascular endothelium, 0 = not found, - = negative, ± = weak, + = moderate reaction, ++ = distinct reaction.

Table 10: Distribution of binding sites of FITC-lectins in adult bovine epididymis.

Lectin	Segment	AC	BC	PC				Lumen	BM	PMC	CT	VE
				St	A.Cyt.	GZ	B. Cyt.					
GSA-I	I	0	±	++	±	±	±	-	-	-	-	+
	II	-	-	++	±	±	±	-	-	-	-	+
	III	-	-	±	-	-	-	-	-	-	-	++
	IV	-	-	-	-	-	-	-	-	-	-	++
	V	-	±	-	-	-	-	-	-	-	-	++
	VI	0	±	-	±	-	±	-	-	-	-	++
PNA	I	0	-	±	±	-	-	++	-	-	-	±
	II	-	-	-	-	-	-	++	-	-	-	-
	III	-	-	-	-	-	-	++	-	-	-	±
	IV	-	-	±	±	±	-	++	-	-	-	-
	V	-	-	±	±	±	-	++	-	-	-	-
	VI	0	-	-	-	-	-	++	-	-	-	-
ECA	I	0	-	+	+	±	-	+	-	-	+	-
	II	-	-	+	+	±	-	±	-	-	±	+
	III	-	-	-	-	-	-	+	-	-	±	-
	IV	-	-	-	-	-	-	++	-	-	+	++
	V	-	-	-	-	-	-	++	-	-	±	+
	VI	0	-	-	-	-	-	±	-	-	±	±
WGA	I	0	++	++	++	+	±	+	+	-	+	±
	II	-	++	++	++	+	±	++	+	-	±	±
	III	-	++	++	++	+	±	++	+	-	±	±
	IV	-	++	++	++	+	±	++	+	-	±	±
	V	-	++	++	++	+	+	++	+	-	±	±
	VI	0	++	++	++	0	+	++	+	-	-	±

Lectin	Segment	AC	BC	PC				Lumen	BM	PMC	CT	VE
				St	A.Cyt.	GZ	B. Cyt.					
Con A	I	0	++	+	+	0	+	+	-	-	±	±
	II	-	++	+	++	0	++	-	-	-	-	-
	III	-	++	+	++	0	++	-	-	-	-	-
	IV	-	++	+	++	0	++	-	-	-	-	-
	V	-	++	+	++	0	++	-	-	-	-	-
	VI	0	++	±	++	0	++	-	-	-	-	-
LCA	I	0	++	+	+	±	+	-	-	-	-	-
	II	-	++	+	+	-	+	-	-	-	-	-
	III	-	++	++	++	-	+	-	-	-	-	-
	IV	-	++	+	+	-	+	-	-	-	-	-
	V	-	++	+	+	+	+	±	-	-	-	-
	VI	0	++	+	±	-	+	±	-	-	-	-
PSA	I	0	+	++	++	-	±	-	-	±	-	-
	II	-	+	++	++	-	-	-	-	-	-	-
	III	-	+	+	+	+	±	±	-	±	-	-
	IV	-	+	+	+	±	±	±	-	-	-	-
	V	-	+	+	+	+	±	±	-	-	-	-
	VI	0	+	+	+	-	±	±	-	-	-	-
DBA	I	0	-	+	+	++	-	-	-	-	-	-
	II	-	-	+	+	±	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-	-	-
	IV	-	-	-	-	-	-	-	-	-	-	-
	V	-	-	-	-	-	-	-	-	-	-	-
	VI	0	-	-	-	0	-	-	-	-	-	-
HPA	I	0	-	-	++	++	+	-	-	-	-	-
	II	-	-	++	++	++	-	-	-	-	-	-
	III	-	-	+	+	-	-	-	-	-	-	-
	IV	-	+	-	+	+	-	-	-	-	-	-
	V	-	+	-	+	+	-	-	-	-	-	-
	VI	0	-	-	-	-	-	-	-	-	-	-

Lectin	Segment	AC	BC	PC				Lumen	BM	PMC	CT	VE
				St	A.Cyt.	GZ	B. Cyt.					
SBA	I	0	-	-	+	-	±	-	-	-	-	-
	II	-	-	-	+	-	+	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-	+	+
	IV	-	-	-	-	-	±	-	-	-	-	-
	V	-	-	-	-	-	±	-	-	-	-	-
	VI	0	-	-	-	-	±	-	-	-	-	-
VVA	I	0	-	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-	-	-
	IV	-	-	-	-	-	-	-	-	-	-	-
	V	-	-	-	-	-	-	-	-	-	-	-
	VI	0	-	-	-	-	-	-	-	-	-	-
LTA	I	0	-	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-	-	-
	IV	-	-	-	-	-	-	-	-	-	-	-
	V	-	-	-	-	-	-	-	-	-	-	-
	VI	0	-	-	-	-	-	-	-	-	-	-
UEA-I	I	0	-	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-	-	-
	IV	-	-	-	-	-	-	-	-	-	-	-
	V	-	-	-	-	-	-	-	-	-	-	-
	VI	0	-	-	-	-	-	-	-	-	-	-

AC = apical cell, A. Cyt. = apical cytoplasm, BC = basal cell, B. Cyt. =basal cytoplasm, BM = basal membrane, CT = connective tissue, GZ = Golgi zone, PC = principal cell, PMC = peritubular muscle coat, St = stereocilia, VE = vascular endothelium, 0 = not found, - = negative, ± = weak, + = moderate reaction, ++ = distinct reaction.

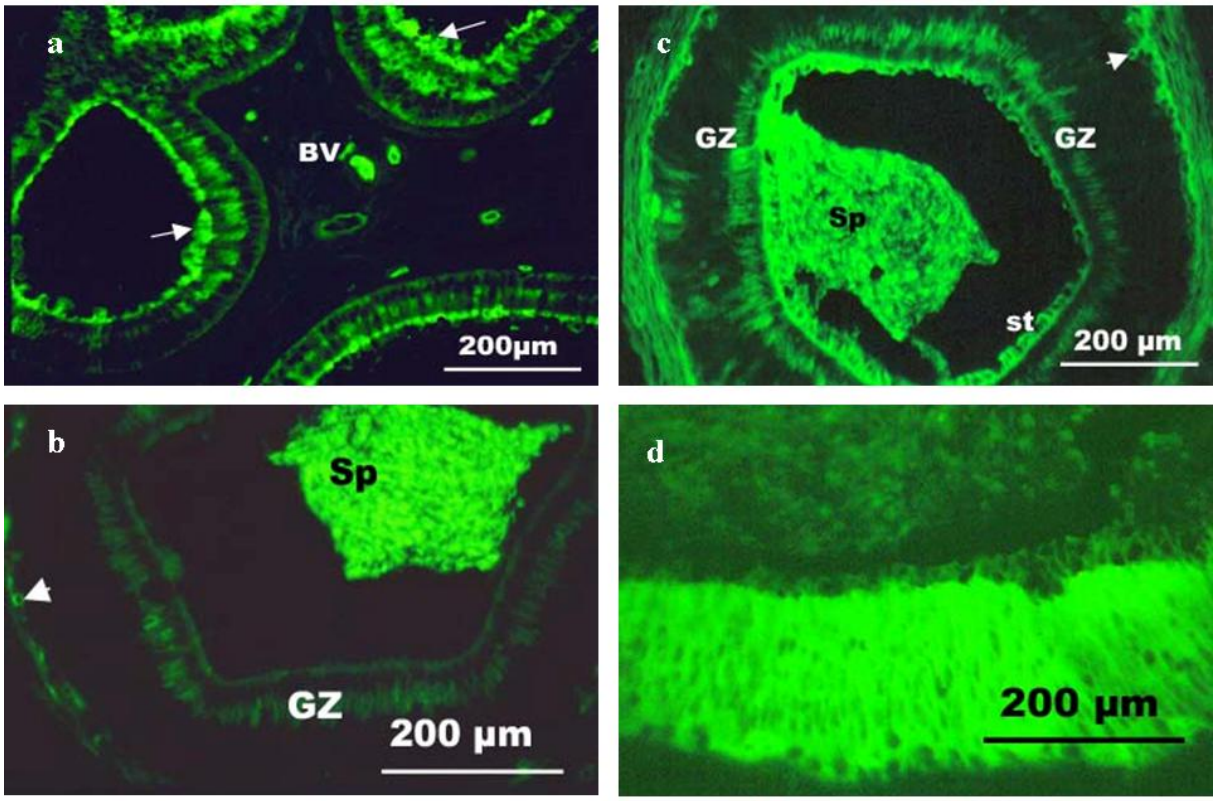


Fig. 19: Distribution of binding sites of FITC-lectins in adult bovine efferent ductules and epididymal duct. GSA-I-binding sites in efferent ductules (a); arrows point to markedly positive apical surface. PNA-binding sites in segment V (b) are mainly restricted to sperm cell mass. WGA-binding sites in segment IV (c). Con A-binding sites in segment VI (d). BV = blood vessels, GZ = Golgi zone, Sp = sperm cell mass, st = stereocilia. Arrow heads point to intraepithelial macrophages.

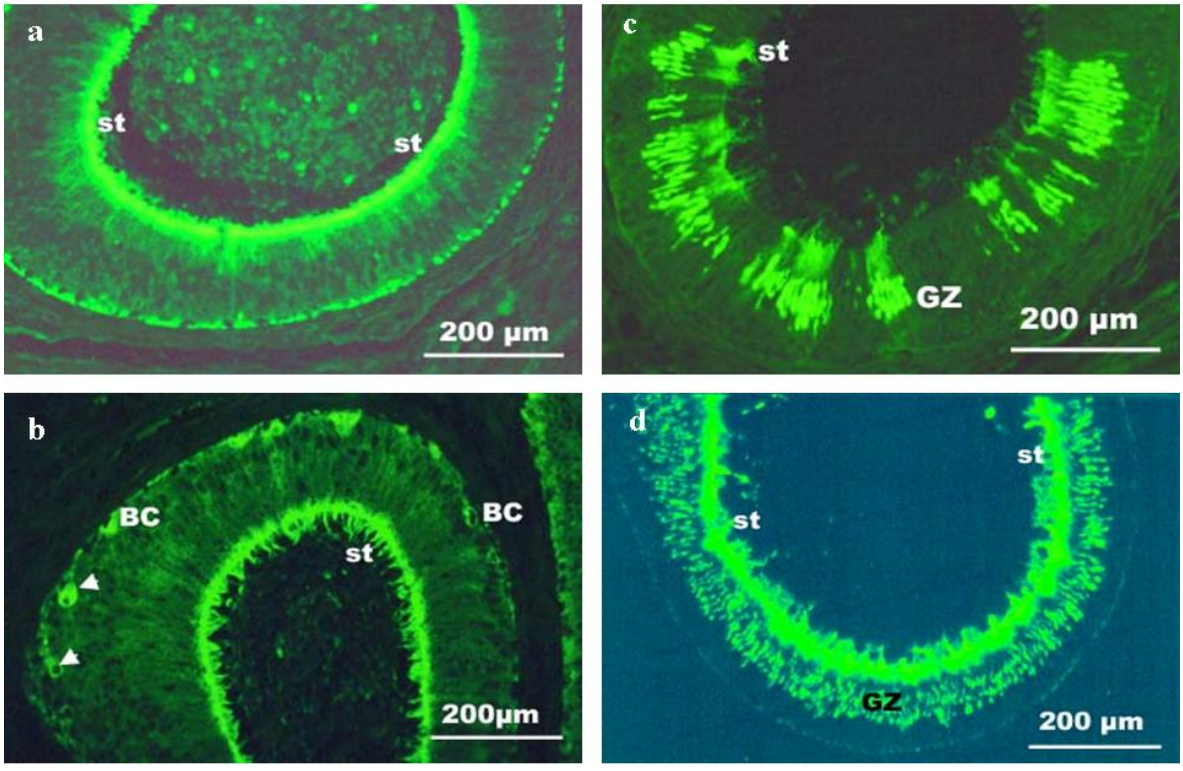


Fig. 20: Distribution of the binding sites of FITC-lectins in the adult bovine epididymis. LCA-binding sites in segment III (a). PSA-binding sites in segment II (b). DBA-binding sites in segment I (c). HPA-binding sites in segment II (d). GZ = Golgi zone, st = stereocilia, BC = basal cells. Arrow heads point to intraepithelial macrophages.

4.2.3 Immunohistochemical studies on the bovine epididymis (Table 11, 12)

4.2.3.1 Distribution of S100 immunoreactivity

The immunoreaction for S-100 revealed variation between the different segments. ED express an alternative pattern of reactivity between the negative nonciliated and the distinctly positive ciliated cells (Fig. 21). Cytoplasm and nuclei of the ciliated cells are positive. Intraepithelial leukocytes are always negative for S100. Apart from some positive cells, which are often located near negative to weakly reactive blood vessels, connective tissue was also negative.

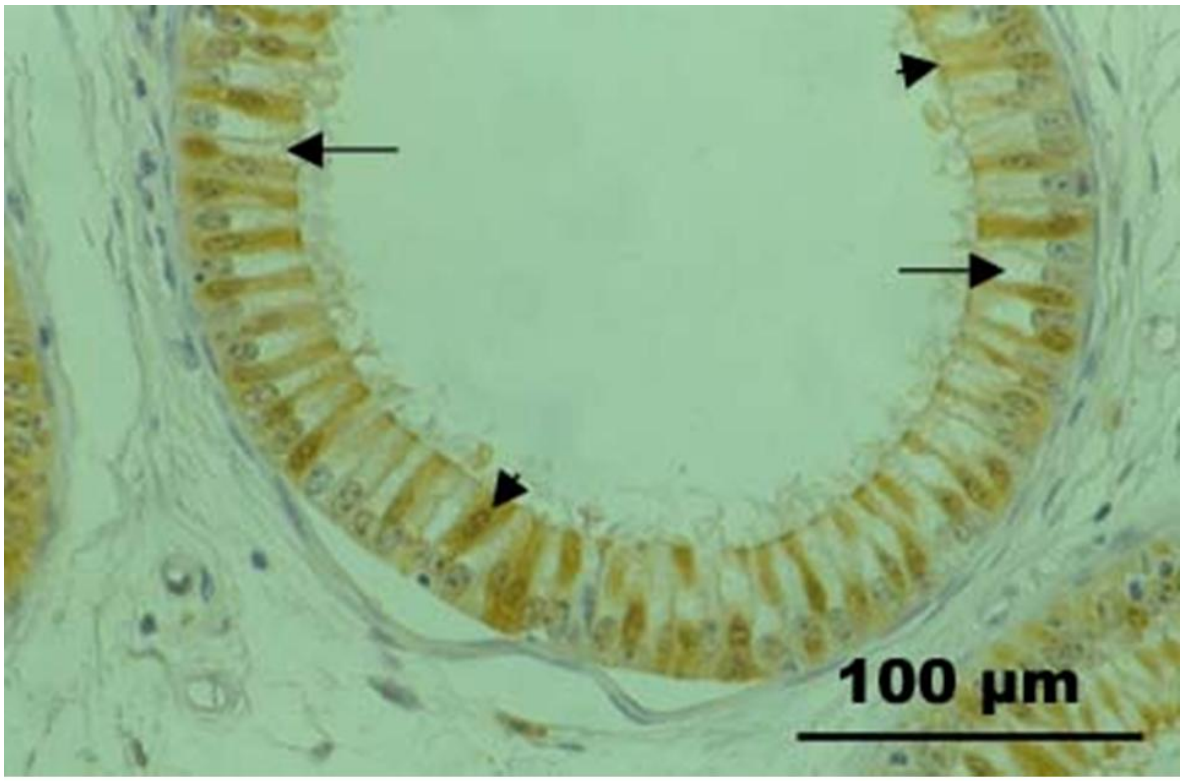


Fig. 21: Immunolocalization of S-100 proteins in efferent ductules of adult bull. Arrows point to negative nonciliated type III cells. Arrow heads point to markedly positive ciliated cells.

Epithelium of the first segment of the epididymal duct shows a positive reaction which becomes stronger at the apical surface and at the openings of epithelial crypts (Fig. 22).
The nuclei of the PC show variable S100-immunoreactivity. Most of them are strongly positive, however along with the positive ones, some negative nuclei are found in the same cross section. Nuclei of the BC and the intraepithelial leukocytes are negative (Fig. 23). Endothelium of blood and lymph vessels and some scattered cells in the connective tissue displayed a weak to moderate immunoreaction. The second and third segments express a pattern of S100-immunoreactivity similar to that encountered in the first segment. Furthermore, AMRC exhibit no S100-immunoreaction neither in nucleus nor in cytoplasm

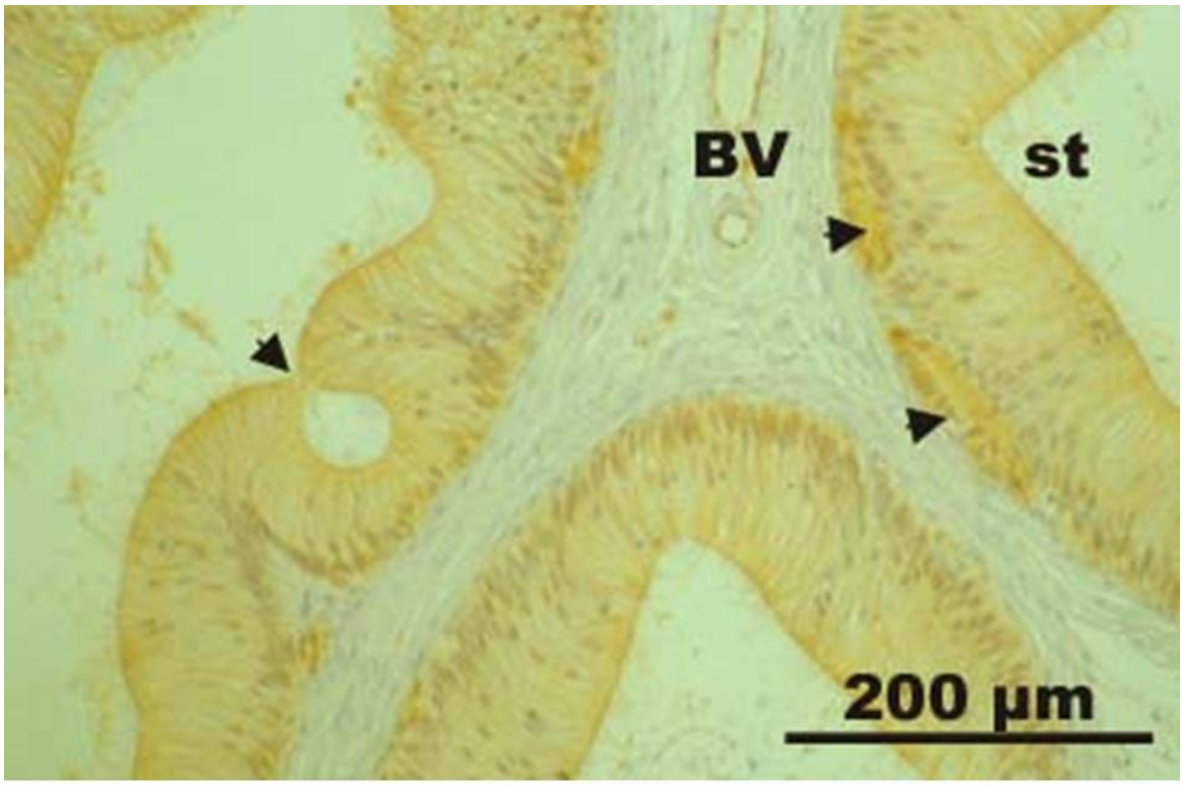


Fig. 22: Immunolocalization of S-100 proteins in segments I of epididymal duct of adult bull. Arrow heads point to distinctly positive epithelial crypts. BV = blood vessels, st = stereocilia.

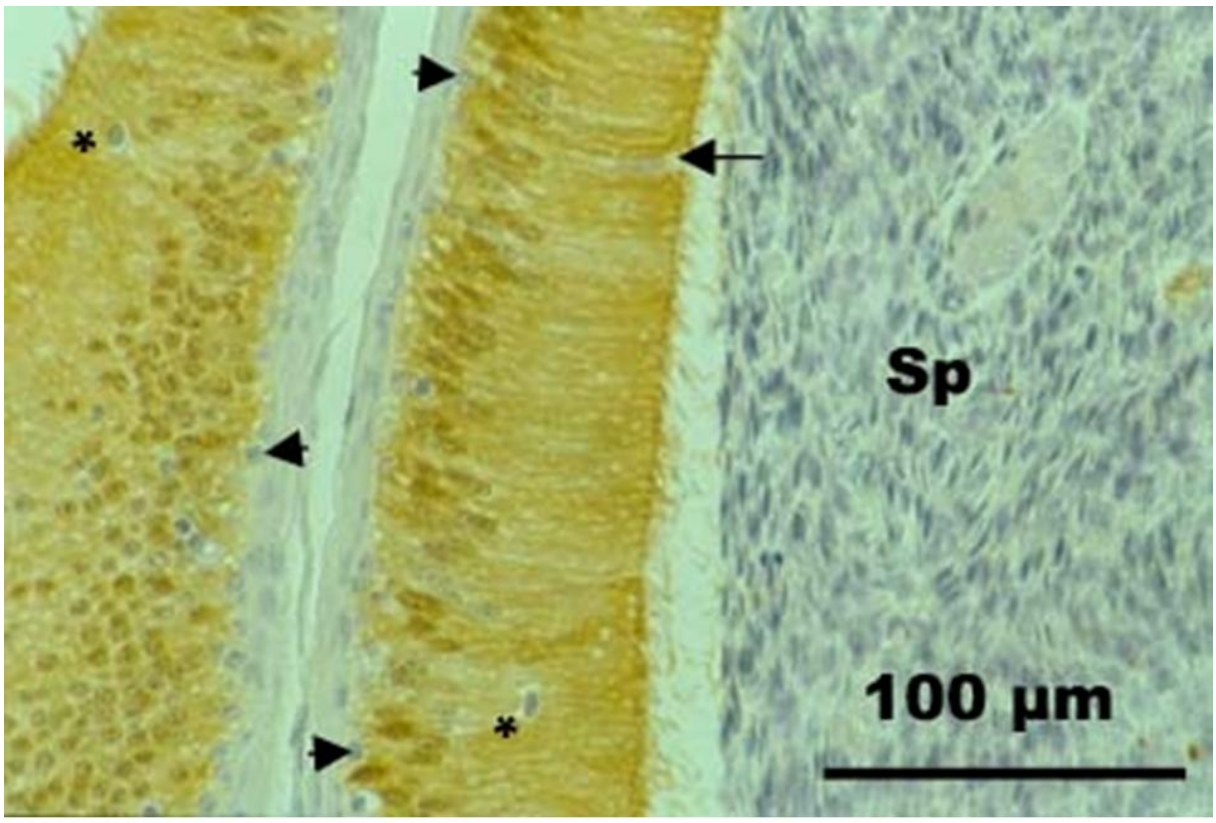


Fig. 23: Immunolocalization of S-100 proteins in segments III of adult bovine epididymal duct. Sp = spermatozoa. Arrow points to negative AMRC. Arrow heads point to negative basal cells. Asterisk points to negative intraepithelial leucocytes.

(Fig. 23). The fourth segment reveals a pattern of reaction similar to that of the preceding segments, but with less intensity. The fifth and sixth segments are negative except some sporadic positive nuclei of the PC, endothelium of blood and lymph vessels and sporadic heteromorphic cells in the connective tissue.

4.2.3.2 Distribution of acidic Fibroblast Growth Factor (aFGF)

A variation in the immunoreactivity for aFGF (FGF-1) is evident between the different regions of the epididymis. The epithelium of the ED exhibits alternating strongly positive ciliated cells and partially positive nonciliated cells (Fig. 24). The latter present a negative supranuclear zone, this occupies about two-thirds of the cell, whereas the basal third shows a positive cytoplasm. On the other hand, the ciliated cells are entirely positive, including both cytoplasm and nuclei. The peritubular SMC are negative. Similarly, the intraepithelial leukocytes are negative for FGF-1. The epithelium of the first three segments of the epididymal duct (caput epididymidis) exhibits a strongly positive basal region surrounding negative nuclei. However, the positive granules are coarser in the second and third segments (Fig. 25). AMRC show a distinctly positive immunoreaction for FGF-1 (Fig. 25, 26).

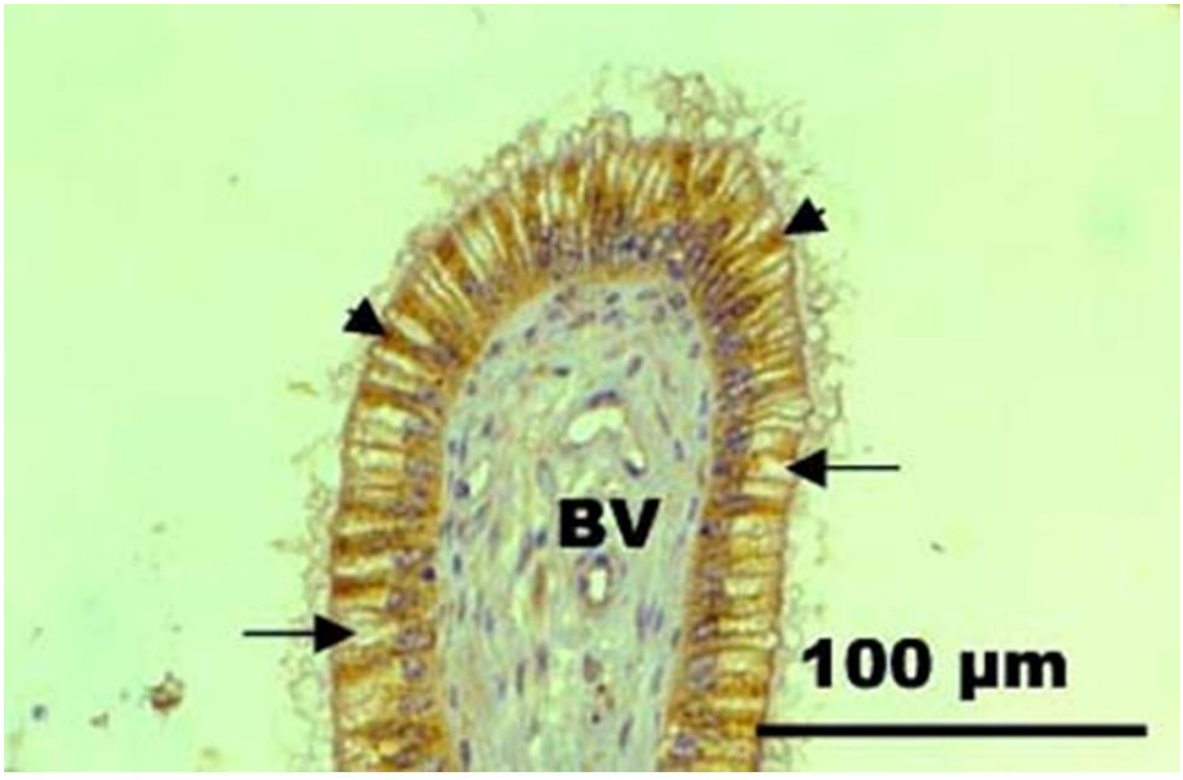


Fig. 24: Immunolocalization of FGF-1 in adult bovine efferent ductule. BV = blood vessels, arrow heads point to distinctly positive ciliated cells, arrows point to negative nonciliated cells.

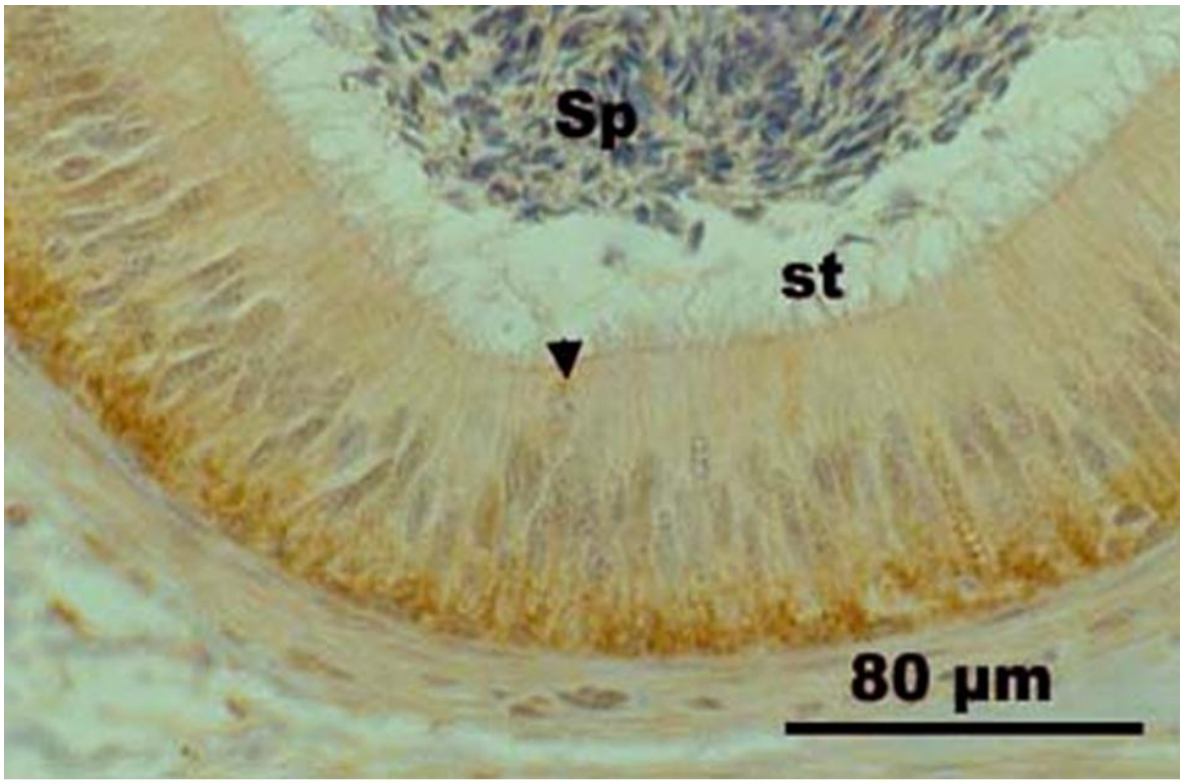


Fig. 25: Immunolocalization of FGF-1 in epididymal segment III. Sp = spermatozoa, st = stereocilia. Arrow heads point to distinctly positive AMRC. Note the intensely positive granules in the basal region of the epithelium.

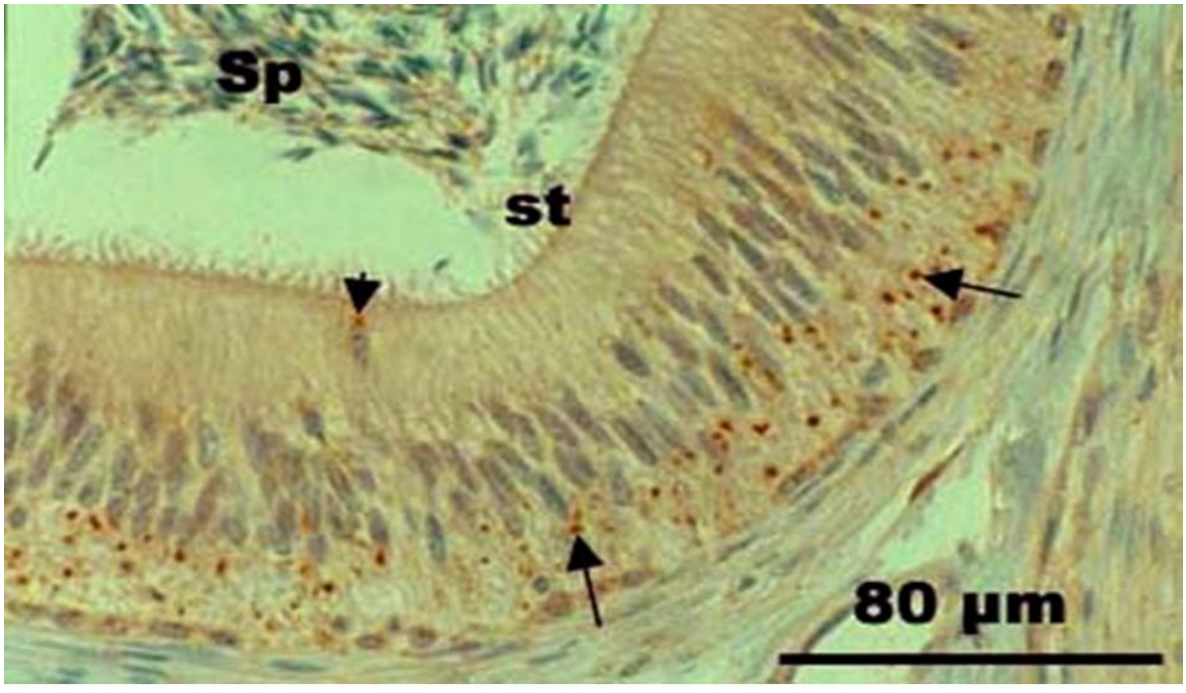


Fig. 26: Immunolocalization of FGF-1 in epididymal segment IV. Sp = spermatozoa, st = stereocilia. Arrow heads point to distinctly positive AMRC, arrows point to intensely positive granules in principal cells.

Both of the fourth and fifth segments possess similar strongly positive granules but they are coarser and loosely distributed throughout the basal third of the cytoplasm (Fig. 26). The

basal portion of the epithelium of the sixth segment shows coarse, strongly positive granules but they are less frequent than in the previous segments. Vascular endothelium as well as some heteromorphic cells near from the blood vessels express a variable immunoreactivity ranges from weak to distinctly positive.

4.2.3.3 Distribution of basic Fibroblast Growth Factor (bFGF)

The immunoreactivity for bFGF (FGF-2) in the bovine ED is represented by a weakly positive cytoplasm as well as strongly positive nuclei of the ciliated cells (Fig. 27). On the other hand, both of the nonciliated cells and intraepithelial leucocytes are negative. The nuclei of the fibroblasts in the lamina propria and periductular SMC express a moderate to distinct FGF-2-immunoreaction (Fig. 27). The nuclei of the vascular endothelial cells exhibit a moderate to strong immunostaining. The interstitial cells, particularly fibroblasts and mast cells possess moderate to strongly positive nuclei. Some cells, probably intraepithelial leucocytes, are negative for FGF-2 (Fig. 27).

Apart from some minor differences, the epithelium lining the different epididymal segments shows a similar immunoreactivity for FGF-2. A strong signal in the nuclei of the BC is found along the length of the duct (Fig. 28), except the sixth segment where BC react somewhat weaker. In contrast to the BC, both of the PC and intraepithelial leucocytes are always negative. The fibroblasts in lamina propria and SMC of the PMC show a reaction pattern similar to those of the ED. In the segments II-V, the nuclei of the AMRC exhibit a variable reactivity that ranges from a negative to strongly positive.

4.2.3.4 Distribution of Angiotensin Converting Enzyme (ACE)

The immunoreactivity for ACE in the ED is strong as expressed by the apical surface and the supranuclear area of many nonciliated cells (Fig. 29). The vascular endothelium of the peritubular as well as the interstitial blood vessels shows a strong ACE-immunoreaction.
The first two segments of the epididymal head demonstrate strongly reactive apical cytoplasm and stereocilia (Fig. 30, 31). Vascular endothelium is similar to those of the ED.
The epithelium of the third segment of the head region as well as those of the body region reveals a negative reaction. Supranuclear cytoplasm of some PC as well as subepithelial blood capillaries of the sixth segment displays a strong immunostaining for ACE (Fig. 32).

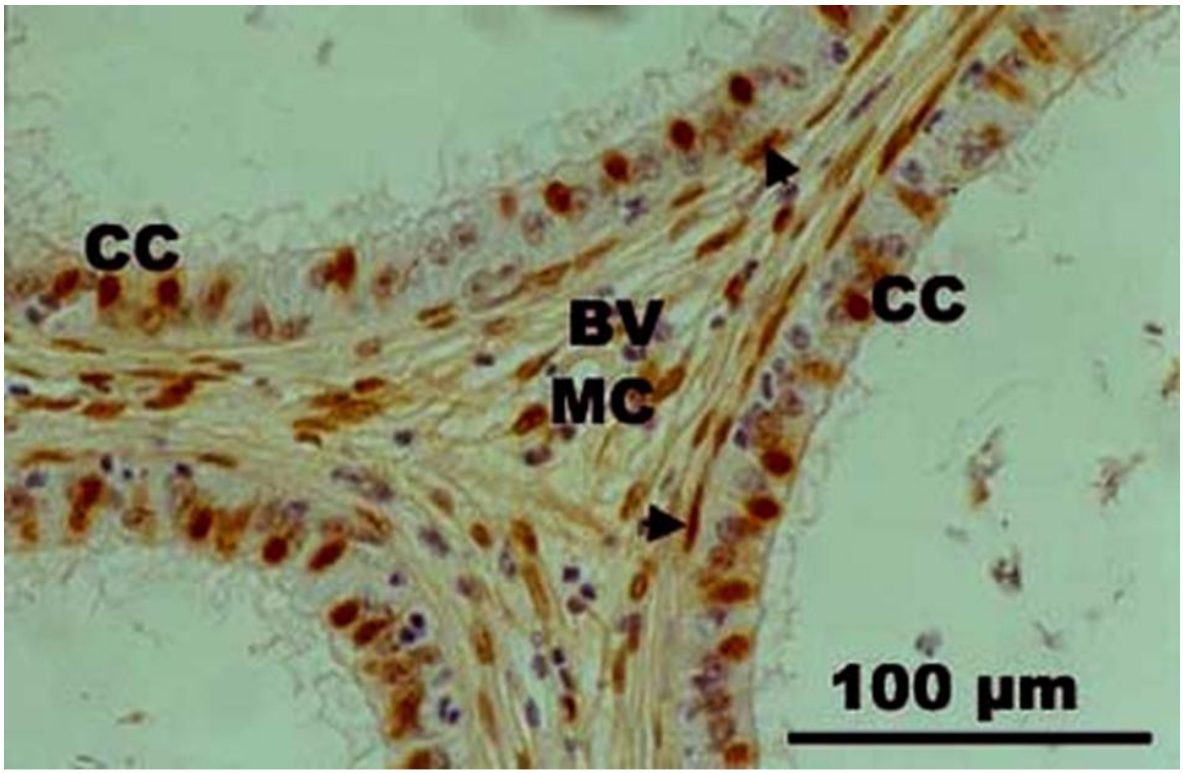


Fig. 27: Immunolocalization of FGF-2 in adult bovine efferent ductules. BV = blood vessels, CC = ciliated cells, MC = mast cells, Arrow heads point to positive fibroblasts in lamina propria.

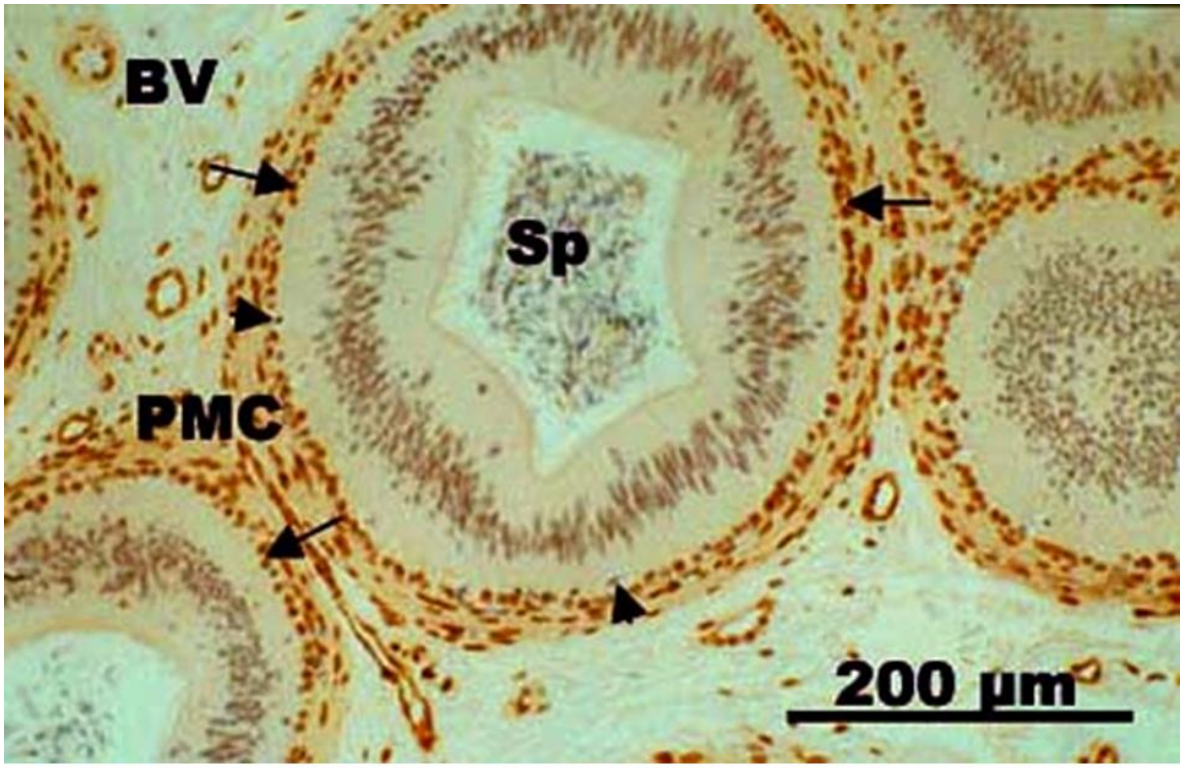


Fig. 28: Immunolocalization of FGF-2 in adult bovine epididymal segment V. BV = blood vessels, CC = ciliated cells, PMC = peritubular muscle coat, Sp = sperm. Arrow heads point to negative intraepithelial leucocytes. Arrows point to intensely positive basal cells.

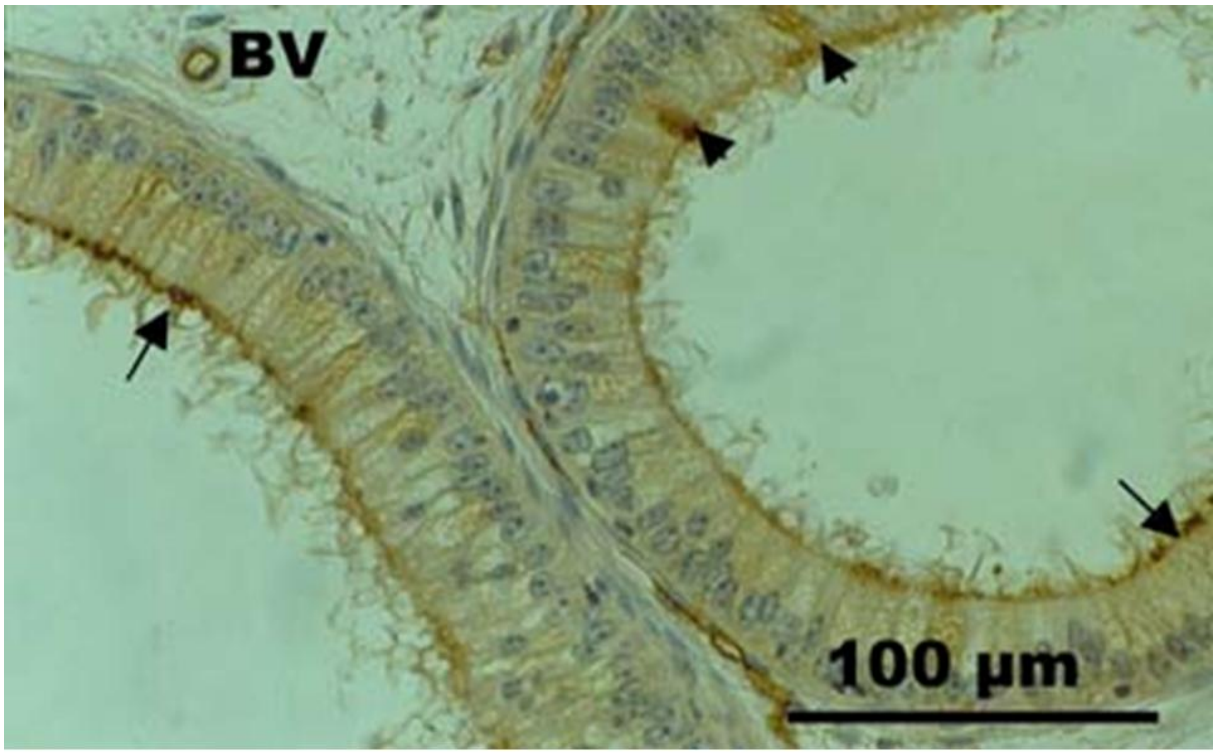


Fig. 29: Immunolocalization of ACE in adult bovine efferent ductules. ACE-immunoreaction is localized on apical surface (arrow) and some nonciliated cells (arrow head) and blood vessels (BV).

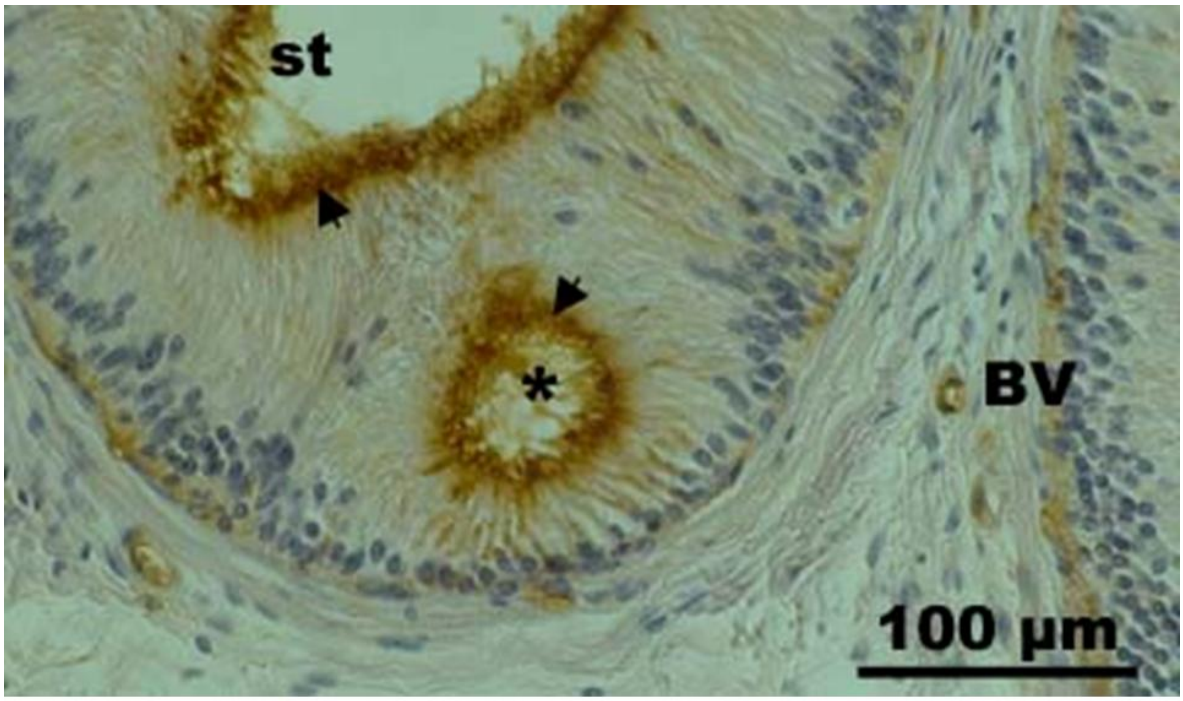


Fig. 30: Immunolocalization of ACE in segment I of adult bovine epididymis. BV = blood vessels, st = stereocilia. Immunoreactivity is localized in strongly positive apical cytoplasm (arrow head) and stereocilia. Asterisk points to an epithelial crypt characteristic for segment I.

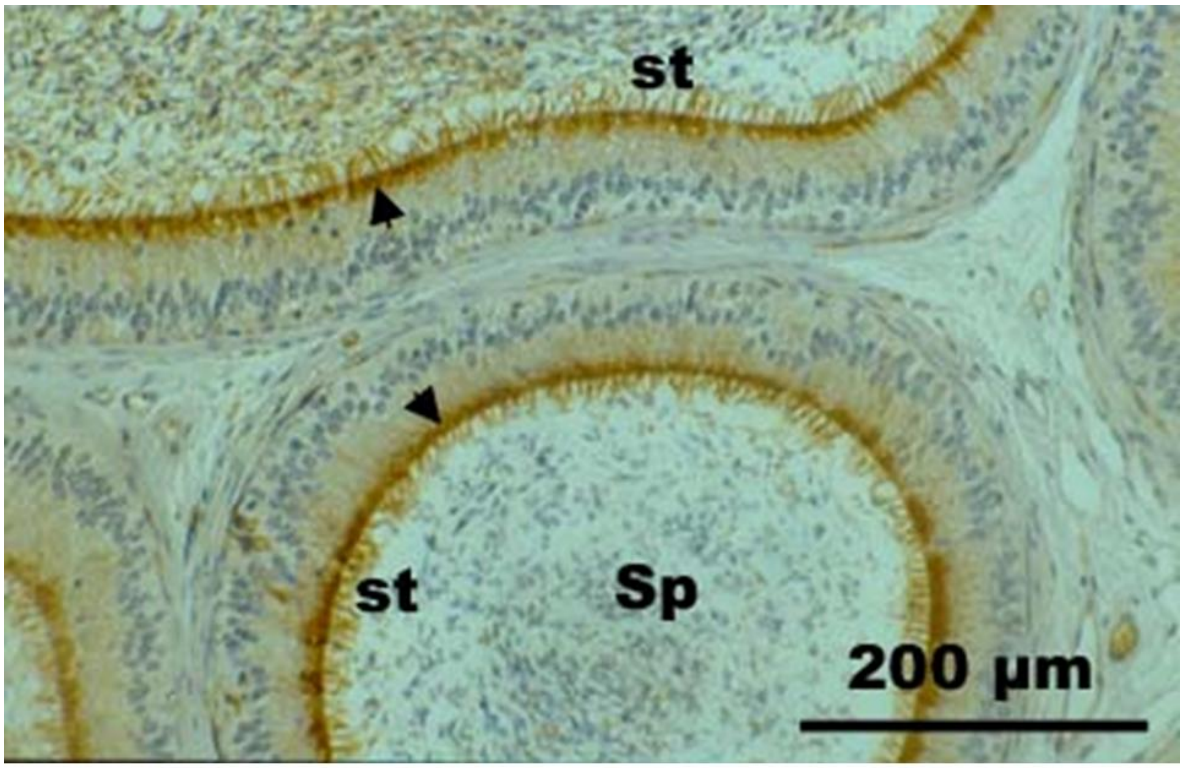


Fig. 31: Immunolocalization of ACE in segment II of adult bovine epididymis. ACE-immunoreaction is localized in strongly positive apical cytoplasm (arrow head) and stereocilia. St = stereocilia, Sp = spermatozoa.

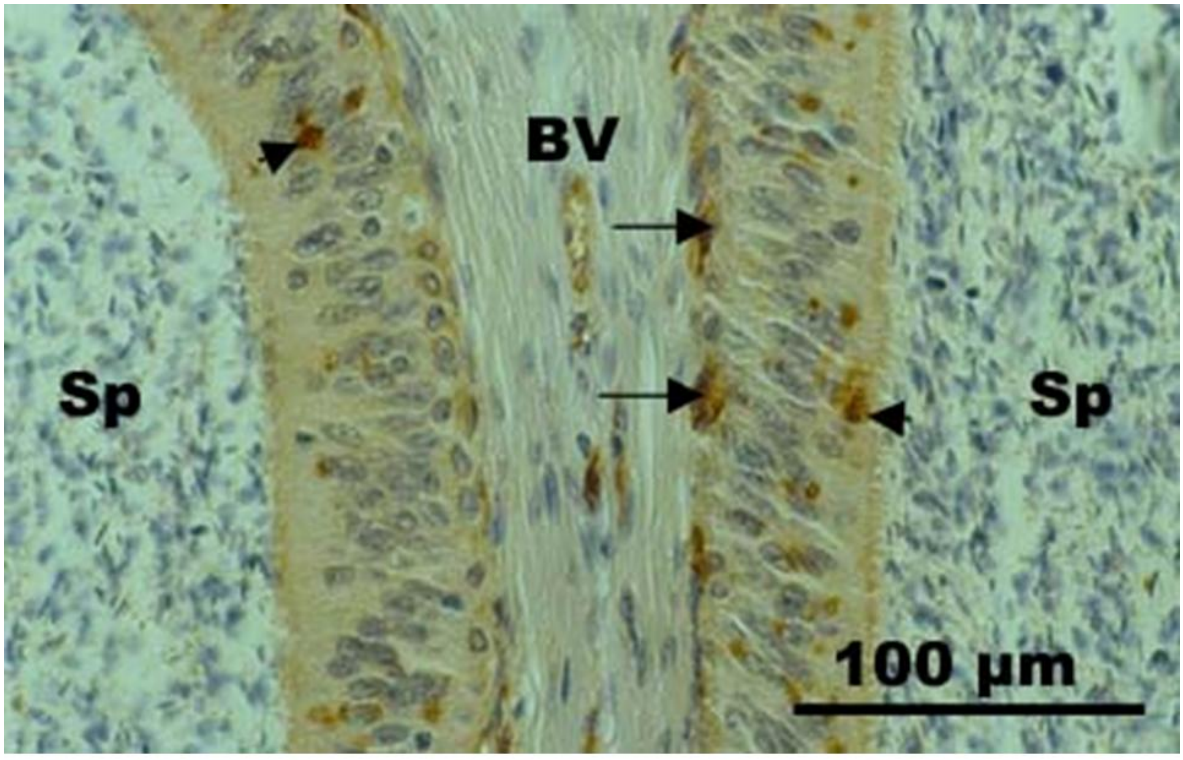


Fig. 32: Immunolocalization of ACE in segment VI of adult bull. Immunoreactivity is confined to supranuclear and apical cytoplasm of some scattered principal cells (arrow head) and to subepithelial capillaries (arrows). BV = blood vessels, Sp = sperm.

4.2.3.5 Distribution of Galactosyltransferase (GT)

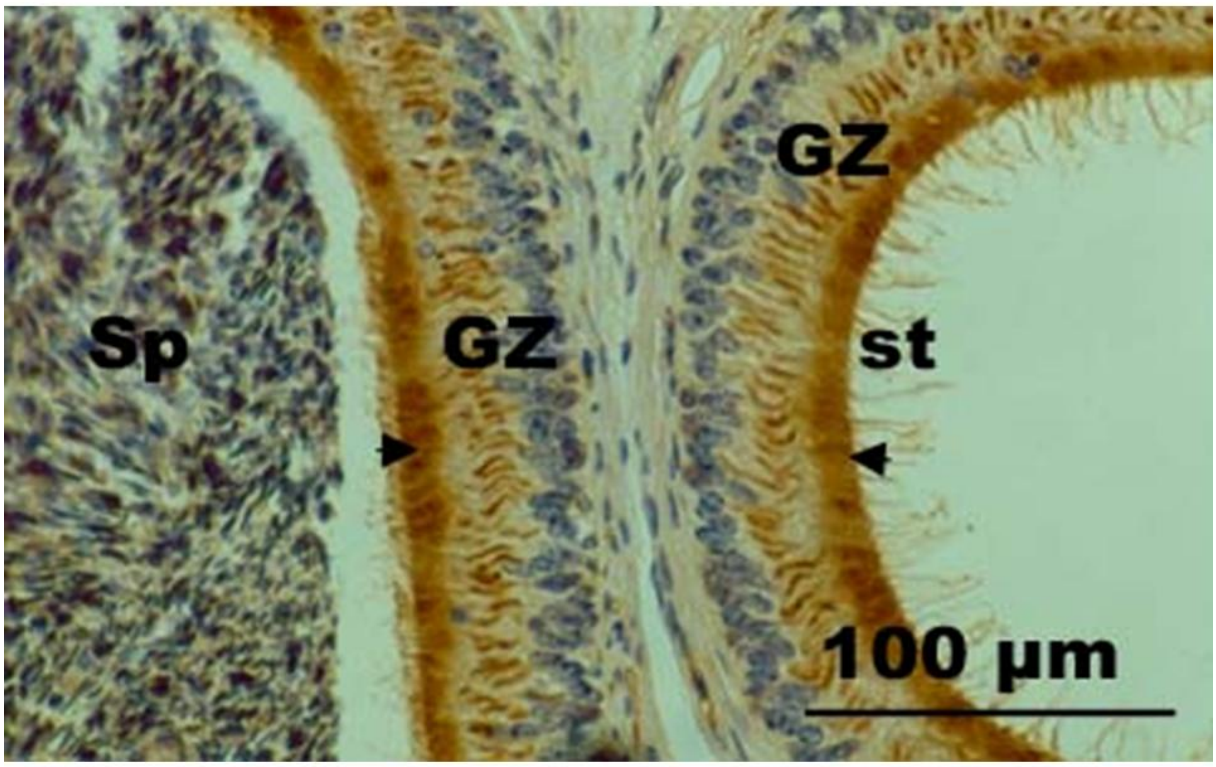


Fig. 33: Immunolocalization of galactosyltransferase in segment II of adult bovine epididymal duct. BV = blood vessels, GZ = Golgolgi zone, Sp = spermatozoa, st = stereocilia. Arrow heads point to strongly positive apical cytoplasm.

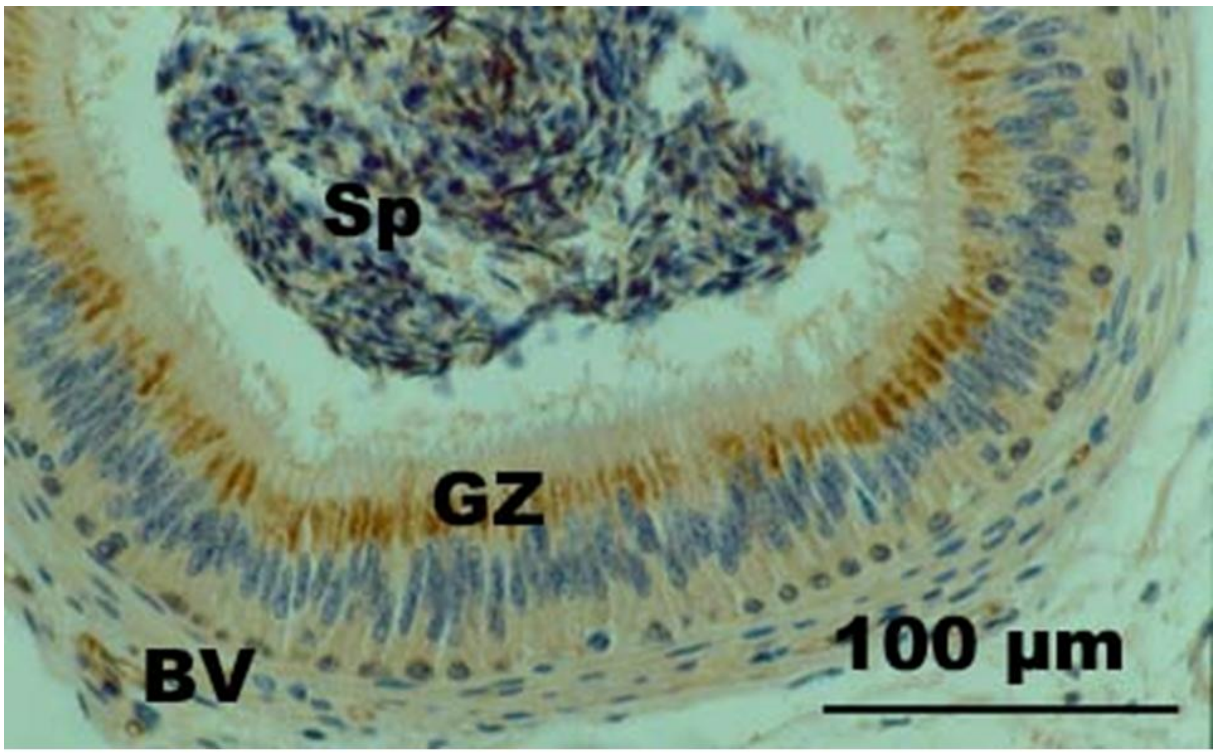


Fig. 34: Immunolocalization of galactosyltransferase in segment III of adult bovine epididymal duct. BV = blood vessels, GZ = Golgi zone, Sp = spermatozoa.

The epithelium lining the ED possesses an apical surface expressing a remarkable immunostaining for GT. Moreover, the supranuclear cytoplasm of the ciliated cells expresses a diffuse weak reaction. The pattern of immunoreactivity for GT shows a marked variation in the different epididymal segments. The GZ of the PC of the epithelium lining the first segment of the epididymal duct exhibited a moderate reaction. Stereocilia of the PC showed a weak to moderate immunoreactivity. The immunoreaction in the second segment is completely different since the PC showed a strongly positive supranuclear GZ and apical cytoplasm as well as weakly reactive stereocilia (Fig. 33). The immunoreactivity for GT in the third segment is similar to that of the second segment but the reaction of the GZ is weaker and that of the apical zone and stereocilia was absent (Fig. 34). The PC in both of the fourth and the fifth epididymal segments possess a weak to moderate GT-immunoreaction in the GZ. The PC of the epithelium lining the sixth segment possess a granular moderate reaction in the supranuclear cytoplasm.

4.2.3.6 Distribution of Vascular Endothelial Growth Factor (VEGF)

The epithelium, the peritubular and the interstitial tissues of the ED and the epididymal duct show no immunostaining for VEGF. However, mast cells in the interstitium express variable

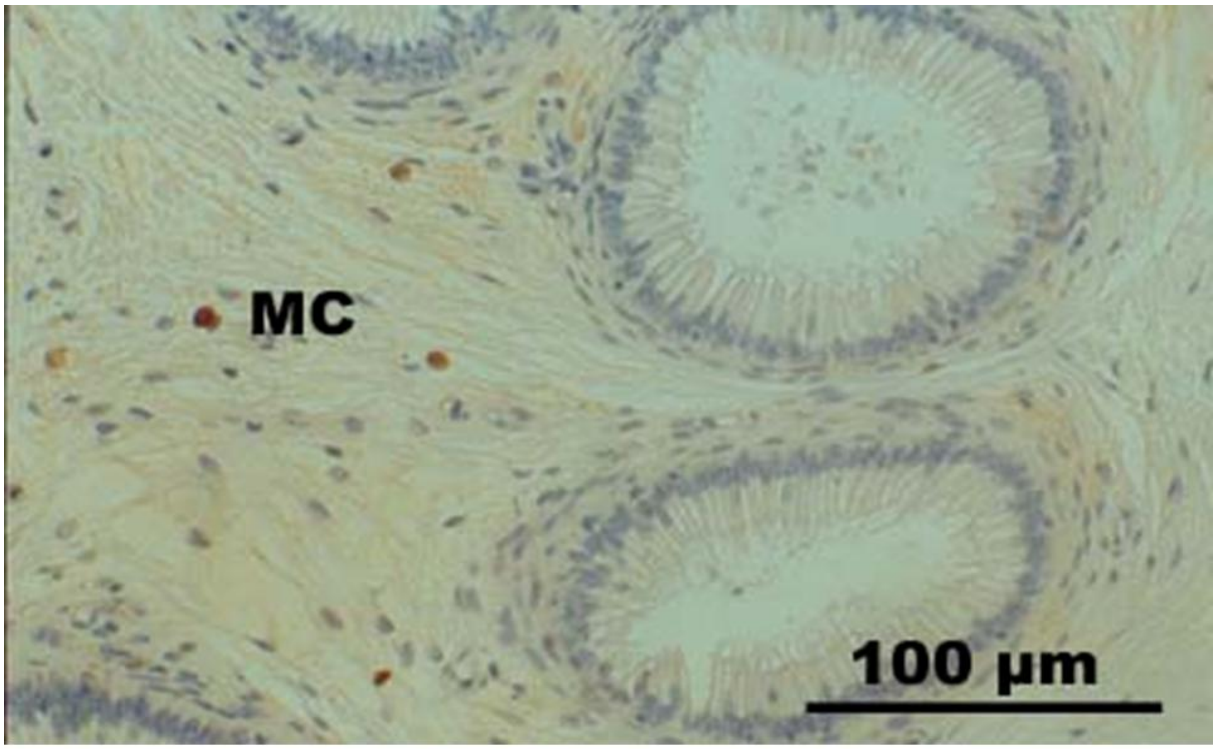


Fig. 35: Immunolocalization of VEGF in ED of adult bull. VEGF-immunoreaction is confined to mast cells (MC).

VEGF-immunoreactivity that ranged from weak to strong positive reaction. Positive cells are frequently found next to blood vessels. The number of the strongly reactive mast cells is the highest in the region of ED (Fig. 35) and in the sixth epididymal segment.

4.2.3.7 Distribution of α-Smooth Muscle Actin (α-SMA)

The cytoplasm of periductal and vascular smooth muscle cells show a strong positive α-SMA-immunostaining. There is no variation in the intensity of the immunoreactivity, neither between the ED (Fig. 36a) and the epididymal duct, nor among the different segments of the epididymal duct (Fig. 36b, 36c).

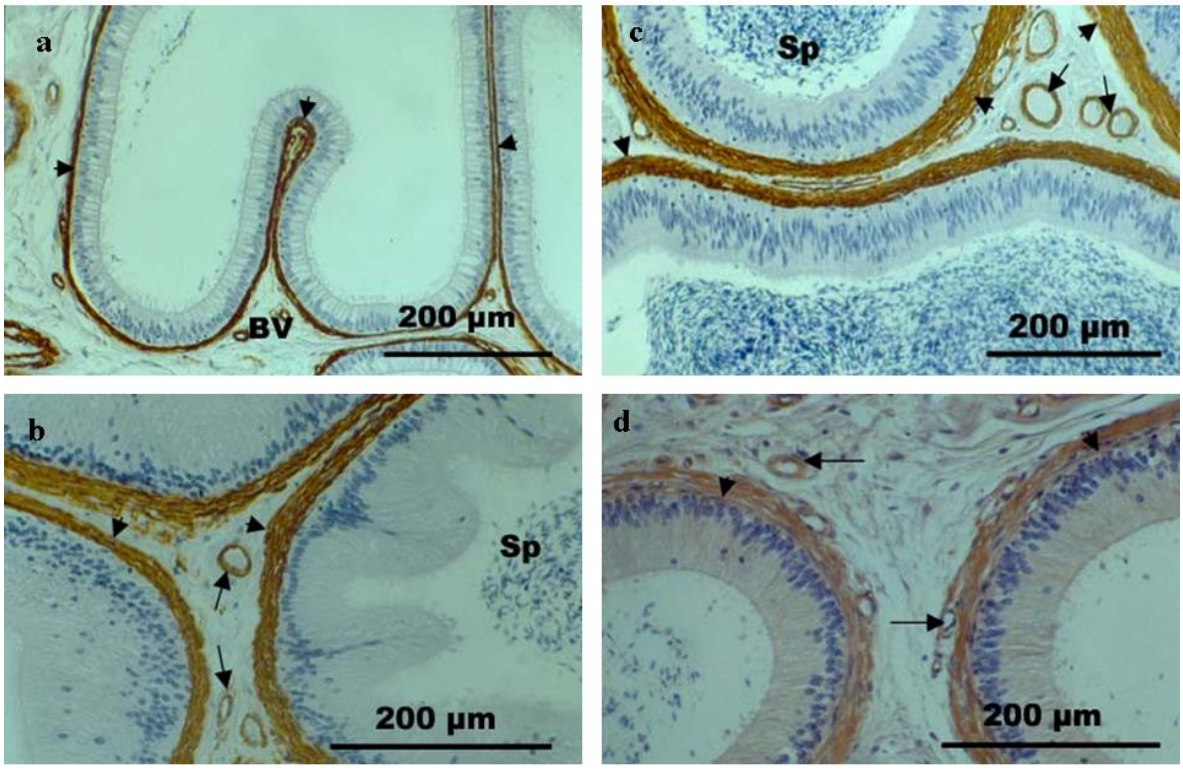


Fig. 36: Immunolocalization of α-SMA in adult bovine efferent ductules (a) and epididymal segments I (b) and VI (c). BV = blood vessels, Sp = spermatozoa. Arrows point to immunreaction in vascular musculature. Arrow heads point to immunoreaction in PMC. Immunolocalization of laminin in the epididymal segment II (d). Arrows point to immunoreaction in vascular endothelium. Arrow heads point to immunoreaction in epithelial basal lamina and in the basal lamina of the smooth muscle cells.

4.2.3.8 Distribution of Laminin

The basal lamina of the epithelium expressed a moderate to distinct immunoreactivity along the whole length of the ED and the epididymal duct. Similarly the basal lamina of the SMC forming the PMC also reacted moderately. The vascular endothelium of the subepithelial capillaries and interstitial blood vessels exhibited a moderate to strong immunoreactiveity for laminin throughout the entire length of the duct (Fig. 36d).

4.2.3.9 Distribution of Connexin 43

There is no immunostaining for connexin 43, neither in ED nor in epididymal duct.

4.2.3. 10 Distribution of T-Lymphocyte markers

CD8, a marker for the T-cytotoxic lymphocytes shows no reactivity in the ED or in any of the epididymal segments, neither intraepithelial nor interstitial. On the other hand, CD4, a marker for the T-helper lymphocytes, shows a moderate to distinct positive reaction in the ED and throughout the entire length of the epididymal duct. The reactive cells are found at different levels of the epithelium, but more frequently near the basal membrane (Fig. 37a, 37b). Similar positive cells have been encountered in the interstitium and rarely intraluminal (Fig. 37a, 37b). Regarding the intensity of reaction, there was no significant difference between the different segments.

4.2.3.11 Distribution of macrophage marker CD 68

$CD68^{+}$ cells are mostly seen in the basal region and rarely at higher levels of the epididymal epithelium along the whole length of the duct. Intraluminal positive cells are only seen in

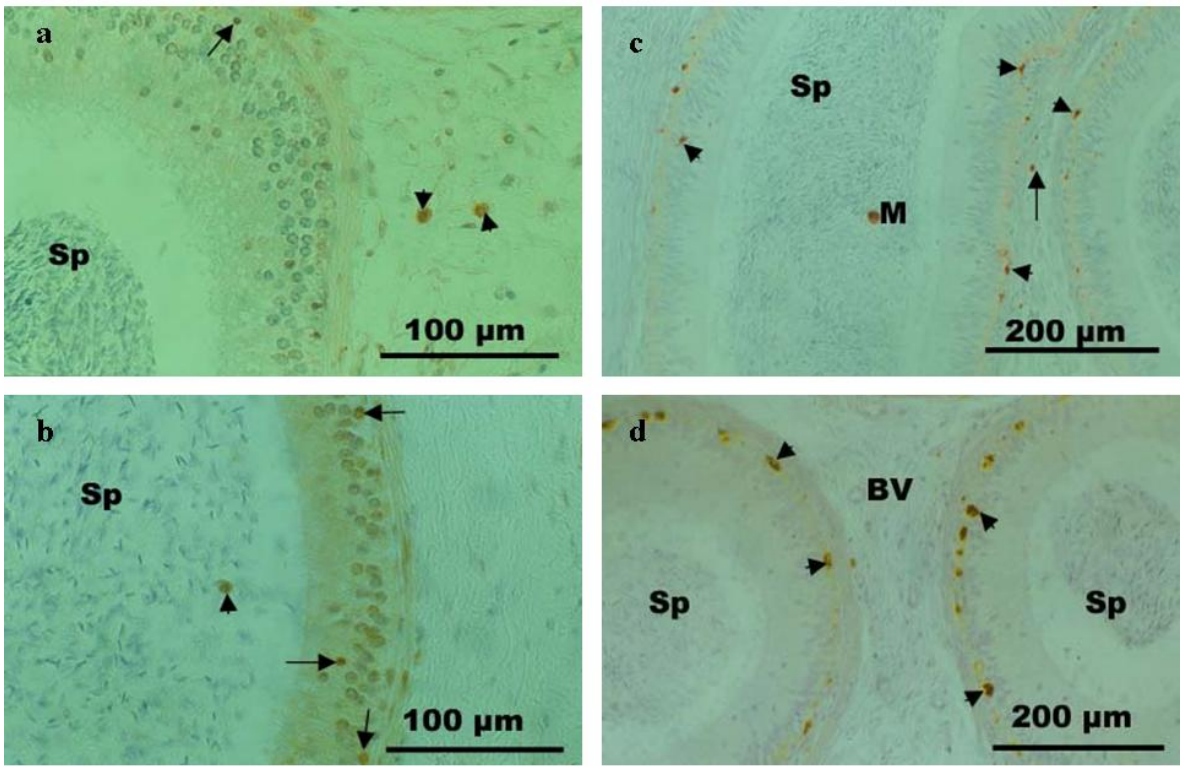


Fig. 37: Immunolocalization of CD4+ positive T-lymphocytes in adult bovine epididymal segment II (a and b). Arrows point to positive intraepithelial lymphocytes. Arrow heads point to positive cells in the interstitium (a) or within the lumen (b). Immunolocalization of $CD68^{+}$ macrophages in adult bovine epididymal segment III (c and d). CD 68+ cells are found intraepithelial (arrow heads), intraluminal (M) and in the interstitium (arrow). BV = blood vessels, M = intraluminal macrophage, Sp = spermatozoa.

some tubules of segment III in one animal (Fig. 37c). The distribution of $CD68^+$ cells was variable in different segments. In segments I, IV and V the number of positive cells ranged from few to moderate. However in segments II, III (Fig. 37c, 37d) and VI the number of the positive cells was higher than in other segments. The number of interstitial $CD68^+$ cells is very low in comparison to that of the intraepithelial cells along the complete length of the duct. In contrast to epididymal segments, the ED contained no intraepithelial $CD68^+$ cells.

Table 11: Distribution of different immunolocalized functional proteins in adult bovine efferent ductules.

Protein	**CC**	**NC**	**IEM**	**IEL**	**BM**	**PMC**	**CT**	**BV/VE**
S-100	++	-	-	-	-	-	-	±
FGF-1	+++	±	-	-	-	-	-	+
FGF-2	+++	-	-	-	-	++	MC	+
ACE	+	+	-	-	-	-	-	+++
GT	+	+	-	-	-	-	-	-
VEGF	-	-	-	-	-	-	MC	-
α-SMA	-	-	-	-	-	+++	-	+++
Laminin	-	-	-	-	++	++	-	+++
Connexin-43	-	-	-	-	-	-	-	-
CD4	-	-	-	++	-	-	L	L^*
CD8	-	-	-	-	-	-	-	-
CD68	-	-	-	-	-	-	-	-

BM = basal membrane, BV = blood vessel, CC = ciliated cell, CT = connective tissue, IEL = intraepithelial lymphocyte, IEM= intraepithelial macrophage, L = interstitial lymphocyte, L^* = intravascular lymphocyte, MC = mast cell, NC = nonciliated cell, VE = vascular endothelium. 0 = not found, ± = weak, + = moderate, ++ = distinct and +++ = strong reaction.

Table 12: Distribution of different immunolocalized functional proteins in the adult bovine epididymis.

Protein	Segment	BC	AC	PC	IEM	IEL	BM	PMC	CT	BV/ VE
S-100	I	-	0	++	-	-	-	-	-	±/+
	II	-	-	++	-	-	-	-	-	±/+
	III	-	-	++	-	-	-	-	-	±/+
	IV	-	-	+	-	-	-	-	-	±/+
	V	-	-	-	-	-	-	-	-	±/+
	VI	-	0	-	-	-	-	-	-	±/+
FGF-1	I	+	0	++	-	-	-	-	-	+
(aFGF)	II	+	+	++	-	-	-	-	-	+
	III	+	+	++	-	-	-	-	-	+
	IV	+	+	++	-	-	-	-	-	+
	V	+	+	++	-	-	-	-	-	+
	VI	+	0	++	-	-	-	-	-	+
FGF-2	I	+++	0	-	-	-	-	++	MC	+
(bFGF)	II	+++	+	-	-	-	-	++	MC	+
	III	+++	+	-	-	-	-	++	MC	+
	IV	+++	+	-	-	-	-	++	MC	+
	V	+++	+	-	-	-	-	++	MC	+
	VI	+++	0	±	-	-	-	++	MC	+
ACE	I	+	0	+++	-	-	-	-	-	+++
	II	±	-	+++	-	-	-	-	-	+++
	III	±	-	-	-	-	-	-	-	+++
	IV	±	-	-	-	-	-	-	-	+++
	V	±	-	-	-	-	-	-	-	+++
	VI	±	0	+++	-	-	-	-	-	+++

Protein	Segment	BC	AC	PC	IEM	IEL	BM	PMC	CT	BV/ VE
GT	I	±	0	+	-	-	-	-	-	-
	II	±	-	+++	-	-	-	-	-	-
	III	±	-	+++	-	-	-	-	-	-
	IV	±	-	++	-	-	-	-	-	-
	V	±	-	++	-	-	-	-	-	-
	VI	±	0	+	-	-	-	-	-	-
VEGF	I	-	-	-	-	-	-	-	MC	-
	II	-	-	-	-	-	-	-	MC	-
	III	-	-	-	-	-	-	-	MC	-
	IV	-	-	-	-	-	-	-	MC	-
	V	-	-	-	-	-	-	-	MC	-
	VI	-	-	-	-	-	-	-	MC	-
α-SMA	I	-	-	-	-	-	-	+++	-	+++
	II	-	-	-	-	-	-	+++	-	+++
	III	-	-	-	-	-	-	+++	-	+++
	IV	-	-	-	-	-	-	+++	-	+++
	V	-	-	-	-	-	-	+++	-	+++
	VI	-	-	-	-	-	-	+++	-	+++
Laminin	I	-	-	-	-	-	++	++	-	+++
	II	-	-	-	-	-	++	++	-	+++
	III	-	-	-	-	-	++	++	-	+++
	IV	-	-	-	-	-	++	++	-	+++
	V	-	-	-	-	-	++	++	-	+++
	VI	-	-	-	-	-	++	++	-	+++
Connexin-43	I	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-
	IV	-	-	-	-	-	-	-	-	-
	V	-	-	-	-	-	-	-	-	-
	VI	-	-	-	-	-	-	-	-	-

Protein	Segment	BC	AC	PC	IEM	IEL	BM	PMC	CT	BV/ VE
CD4	I	-	-	-	-	++	-	-	L	L*
	II	-	-	-	-	++	-	-	L	L*
	III	-	-	-	-	++	-	-	L	L*
	IV	-	-	-	-	++	-	-	L	L*
	V	-	-	-	-	++	-	-	L	L*
	VI	-	-	-	-	++	-	-	L	L*
CD8	I	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-
	IV	-	-	-	-	-	-	-	-	-
	V	-	-	-	-	-	-	-	-	-
	VI	-	-	-	-	-	-	-	-	-
CD68	I	-	-	-	+++	-	-	-	M	M*
	II	-	-	-	+++	-	-	-	M	M*
	III	-	-	-	+++	-	-	-	M	M*
	IV	-	-	-	+++	-	-	-	M	M*
	V	-	-	-	+++	-	-	-	M	M*
	VI	-	-	-	+++	-	-	-	M	M*

AC = apical cell, BC = basalcell, BM = basal membrane, BV = blood vessels, CT = connective tissue, IEL = intraepithelial lymphocyte, IEM= intraepithelial macrophage, L = interstitial lymphocyte, L* = intravascular lymphocyte, M = interstitial macrophage, M* = intravascular macrophage, MC = mast cell, PC = principal cell, VE = vascular endothelium. 0 = not found, ± = weak, + = moderate, ++ = distinct, +++ = strong reaction.

4.2.4 Ultrastructural characteristics of the bovine epididymis

4.2.4.1 Efferent ductules

The epithelium lining the bovine efferent ductules is simple columnar. It consists of ciliated and nonciliated cells, which are two different categories of columnar cells (Fig. 38, 39). Furthermore, intraepithelial lymphocytes and macrophages are also found within the ductular

epithelium. Based upon the cytological characteristics, nonciliated cells could be categorized into three subtypes: type I cells (contain neither granules nor vacuoles), type II cells (abundant in granules) and type III cells (abundance of vacuoles). The nonciliated type I cells possess ultrastructural characteristics, which are also valid for the other two types. The nucleus is less electron-dense than those of ciliated cells. They possess poorly developed Golgi apparatus in juxtanuclear cytoplasm. Endoplasmic reticulum is less developed and long mitochondria are found next to lateral cell membrane and in the infranuclear area. The luminal surface of nonciliated type I cells shows either a uniform microvillous border or an alternation of microvilli and apical protrusions of various sizes. These protrusions contain granules or vacuoles (Fig. 39) and may be found released in the lumen along with granules and vacuoles. Nonciliated type I cells possess an endocytotic apparatus beneath their microvillous border. This apparatus is composed of a variable number of microcanaliculi, micro-pinocytotic vesicles, coated vesicles and vacuoles. The nuclei of nonciliated cells are euchromatic and located in the basal third of the cells. They are less electron-dense than those of ciliated cells. Nonciliated type II cells possess round, mostly homogenous and membrane-bound granules, which almost fill the cytoplasm. The size of granules increases from the basal to luminal parts of the cell. They frequently present variation in shape, number, size and density among different cells or even within the same cell. Granules originate from expanded rough endoplasmic reticulum (rER). Nonciliated type III cells are characterized by occurrence of vacuoles in their supranuclear area (Fig. 38). Vacuoles demonstrate small flocculent and membranous content. They possess a characteristic pattern of arrangement as stacks in a peg-and-socket manner. Mitochondria are arranged in a row along the lateral cell membrane and present typical transverse cristae. Sporadic profiles of rER are encountered. Golgi cisternae enclose pale contents similar to that of the vacuoles.

Ciliated cells are distinguished from nonciliated cells not only by the cilia but also by the apical position of their nuclei, which are ovoid and heterochromatic. Supranuclear cytoplasm contains a small Golgi apparatus, few irregularly-shaped dense bodies and many mitochondria (Fig. 39). The latter are abundant both in the apical and basal regions. Contrary to those of nonciliated cells, mitochondria possess fewer and mostly longitudinally oriented cristae. Endoplasmic reticulum is inconspicuous. The apical surfaces are equipped with typical kinocilia as well as a few microvilli scattered in-between (Fig. 38, 39). Elements of the endocytotic apparatus are ill-developed.

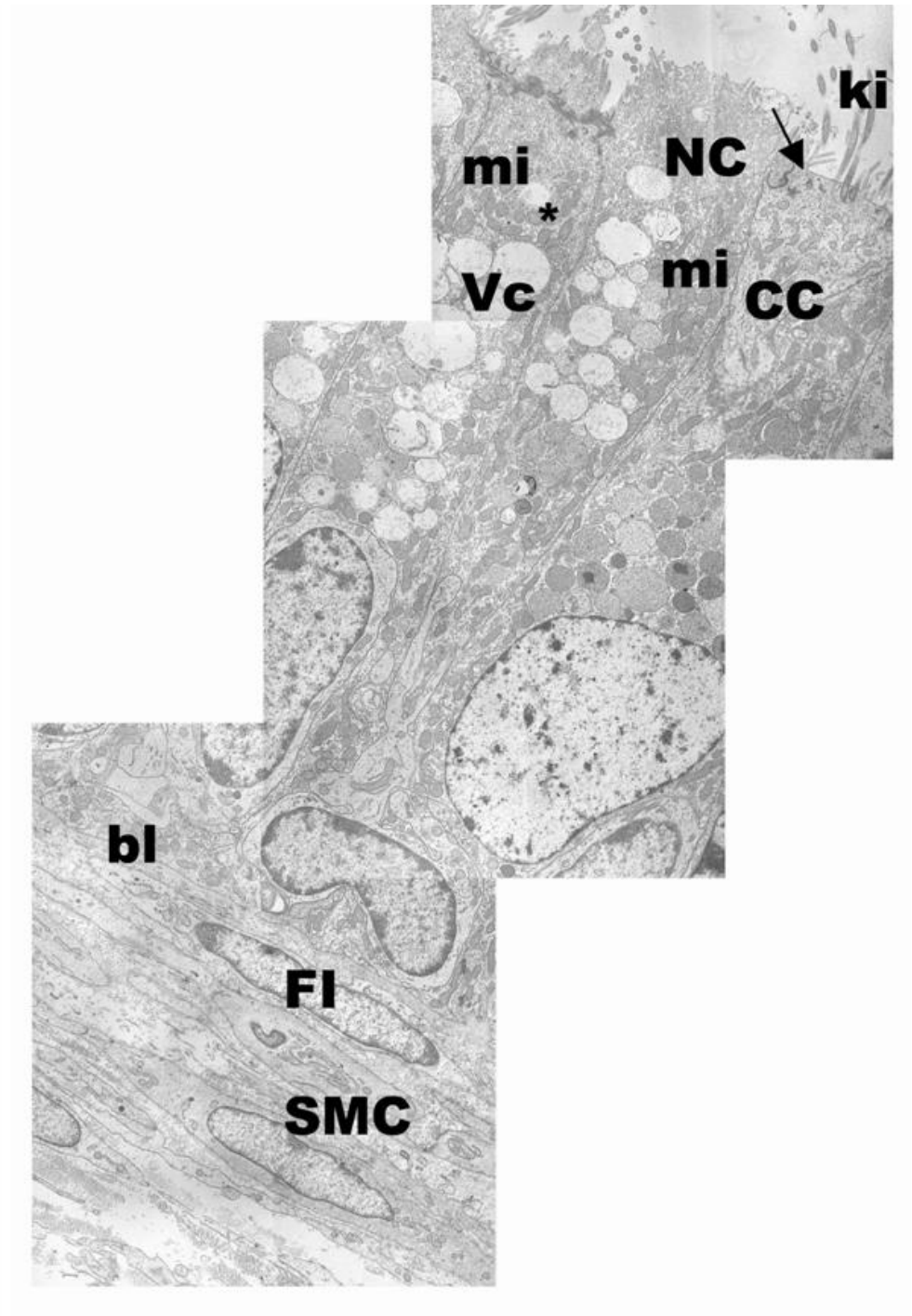


Fig. 38: Electron micrograph of adult bovine efferent ductules. BC = basal cell, bl = basal lamina, CC = ciliated cell, FI = fibroblast, ki = kinocilium, Lu = lumen, mi = mitochondria, NC = nonciliated cell, SMC = smooth muscle cell, Vc = vacuole, arrow points to microvilli, asterisk points to lipid droplet. (x 7000).

Intraepithelial lymphocytes are scattered throughout the epithelium and are characterized by a strongly heterochromatic nuclei and paucity of organelles. Intraepithelial macrophages are found with their pseudopodia insinuating between the epithelial cells and possess a high number of lysosomes and residual bodies.

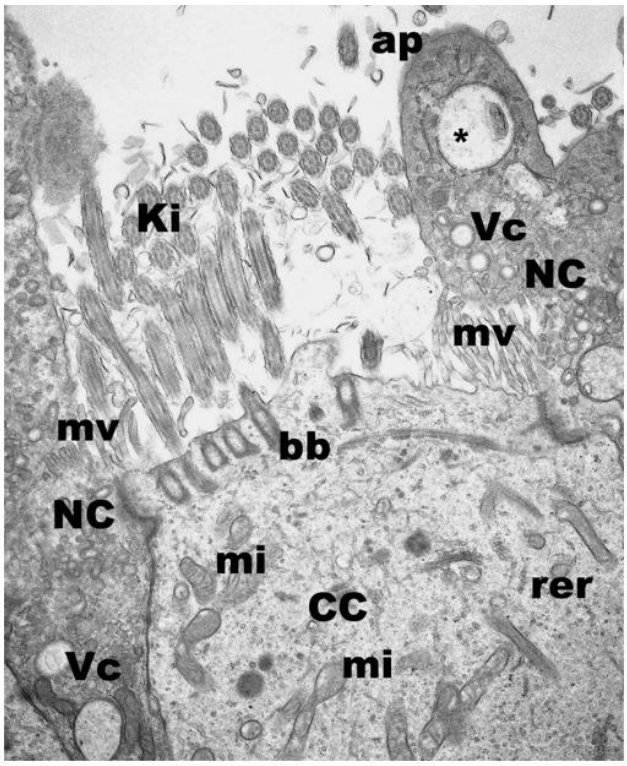


Fig. 39: Electron micrograph of the apical area of the adult bovine efferent ductules. ap = apical protrusion, bb = basal body, CC = ciliated cell, ki = kinocilium, mi = mitochondria, mv = microvillus, NC = nonciliated cell, rer = rough endoplasmic reticulum, Vc = vacuole, asterisk points to a vacuole within the apical protrusion. (x 7000).

4.2.4.2 Epididymal epithelium

The epithelium of the bovine epididymis consists of several cell types. The principal (PC) and basal cells (BC) are the main two cell types that are found along the whole length of the duct. AMRC constitute another cell type, which appear in the apical epithelium of the epididymal segments II-V. Both IEL and IEM are seen in variable numbers in the different epididymal segments.

4.2.4.2.1 Segment I

The high columnar PC are the main cell type of the epididymal epithelium. The apical surfaces of the PC are equipped by long stereocilia. Furthermore, apical cytoplasmic protrusions exist on the luminal surface and contain short strands of smooth endoplasmic reticulum (sER) and few ribosomes and may be found separated into the lumen. Many pinocytotic invaginations are found in the apical cell membrane in the spaces confined between the stereocilia (Fig. 40, 41). The apical cytoplasm contains numerous small smooth-walled and coated vesicles. The content of these vesicles is light or moderate electron dense and occasionally, this material appears as condensed electron dense granules.

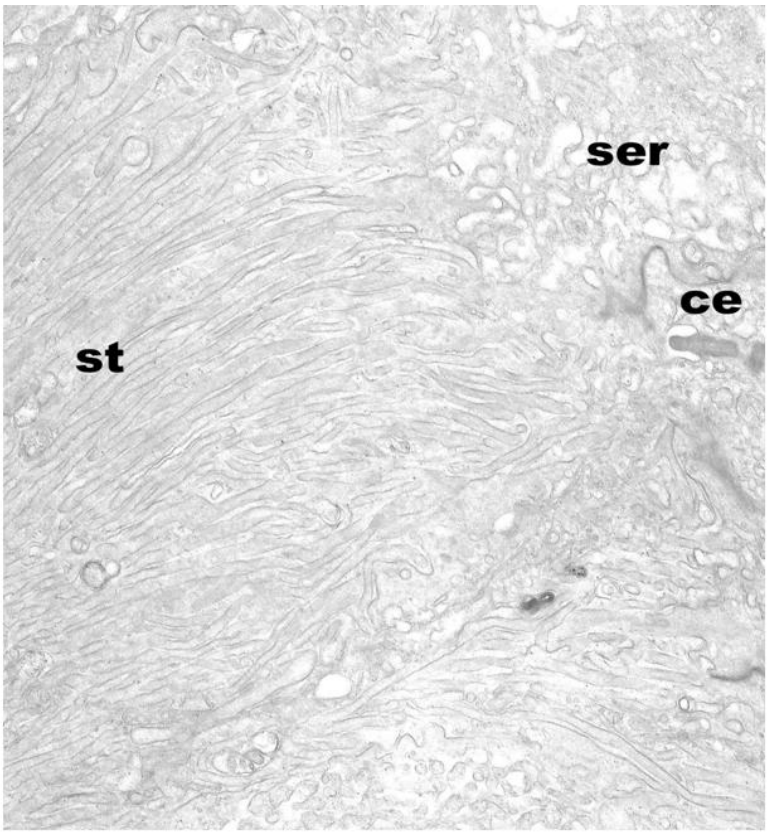


Fig. 40: Apical region of a principal cell in the bovine epididymal segment I. ce = centriol, ser = smooth endoplasmic reticulum, st = stereocilia. (x 7000).

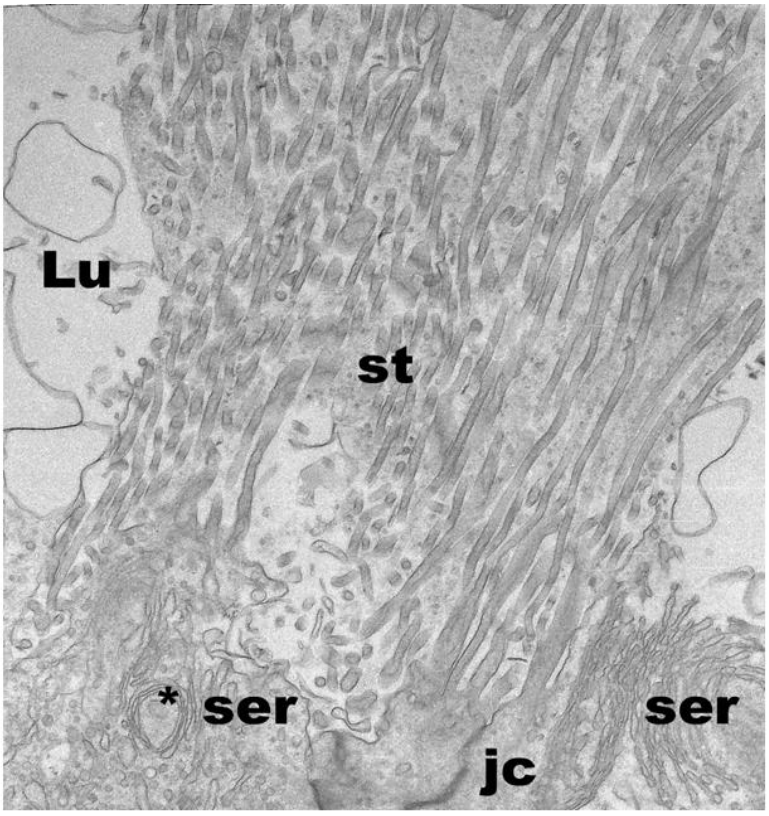


Fig. 41: apical region of a principal cell of bovine epididymal segment I. jc = junctional complex, Lu = lumen, ser = smooth endoplasmic reticulum, st = stereocilia, asterisk points to a lipid droplet surrounded by whorls of smooth endoplasmic reticulum. (x 7000).

Larger vacuoles as well as numerous short profiles of sER and occasionally sparsely granulated ER are found (Fig. 40, 41). However, the length of cisternae and their content of ribosomes increase towards the nucleus. Large numbers of small MVBs exist both in apical and supranuclear cytoplasm. Numerous microtubules are seen between the vesicles and the ER. They are arranged parallel to the long axis of the cell and sometimes, they may be seen in continuity with stereocilia. A well-developed Golgi apparatus dominates in the juxtanuclear zone (Fig. 42). Numerous small vesicles with moderately electron dense content are observed in the centre of this array of Golgi stacks. They evidently bud from the inner cisternae as short smooth-walled cisternae. The longer cisternae are peripherally and concentrically arranged. Furthermore, they are bound through cross-links particularly in the peripheral areas. A large number of rER occurs peripheral to the Golgi complex, particularly close to the lateral cell membrane. The rER cisternae contain moderate electron-dense material. Numerous mitochondria, a few cisternae of rER as well as many small vacuoles present in the infranuclear cytoplasm. The mitochondria predominating in the infranuclear cytoplasm are oval or elongated in shape. In addition to the mitochondria, various forms of numerous small vesicles and small electron dense granules are found in the basal cytoplasm.

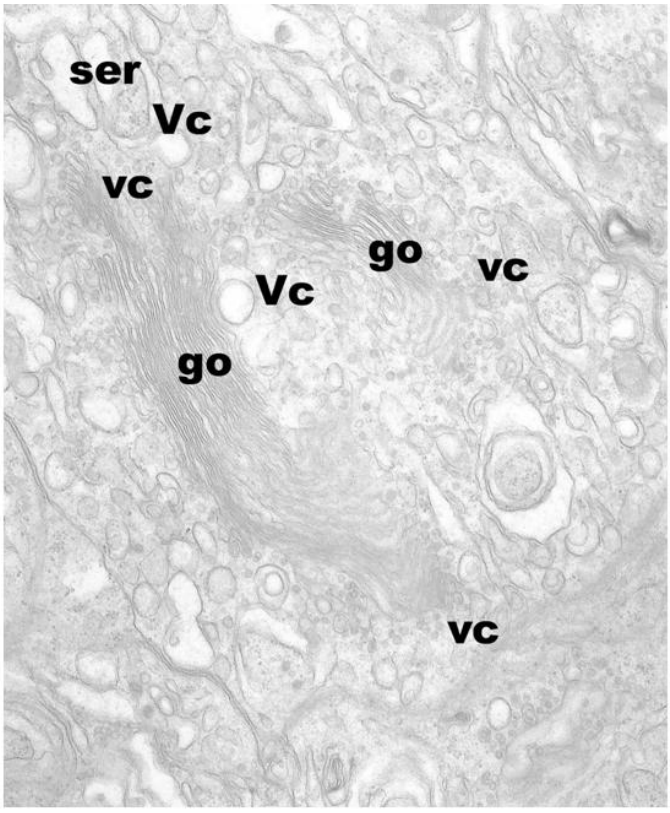


Fig. 42: Electron micrograph showed Golgi apparatus in the supranuclear cytoplasm of adult bovine epididymal segment I. go = Golgi apparatus, ser = smooth endoplasmic reticulum, vc = vesicle, Vc = vacuole. (x 12,000).

The nucleus of the PC is oval in shape and is located in the basal third of the cell. It possesses a moderate content of heterochromatin. The latter is distributed in the form of a narrow peripheral rim as well as small islands throughout the karyoplasm. Many of the nuclei possess one or more compact nucleoli. The basal cells (BC) are small pyramidal cells and never reach the luminal surface. They are insinuated between the narrow basal extensions of the PC. Thus their contact with the basal lamina (BL) is restricted to a small area. Their nuclei are kidney-shaped and are always deeply invaginated or even subdivided in two lobes. In addition to a few number of mitochondria with lamellar cristae, the lightly stained cytoplasm contains a small Golgi apparatus, a few strands of rER and a large number of free ribosomes. Furthermore, the cytoplasm contains bundles of cytoplasmic filaments and lipid droplets. They contact with the neighbouring high prismatic PC through interdigitation. Intraepithelial lymphocytes (IEL) are observed in the epithelium lining the first segment. They possess dense round nuclei and a narrow peripheral cytoplasmic rim. Although they exist in large numbers in the basal area, IEL have been seen at different levels of the epithelium.

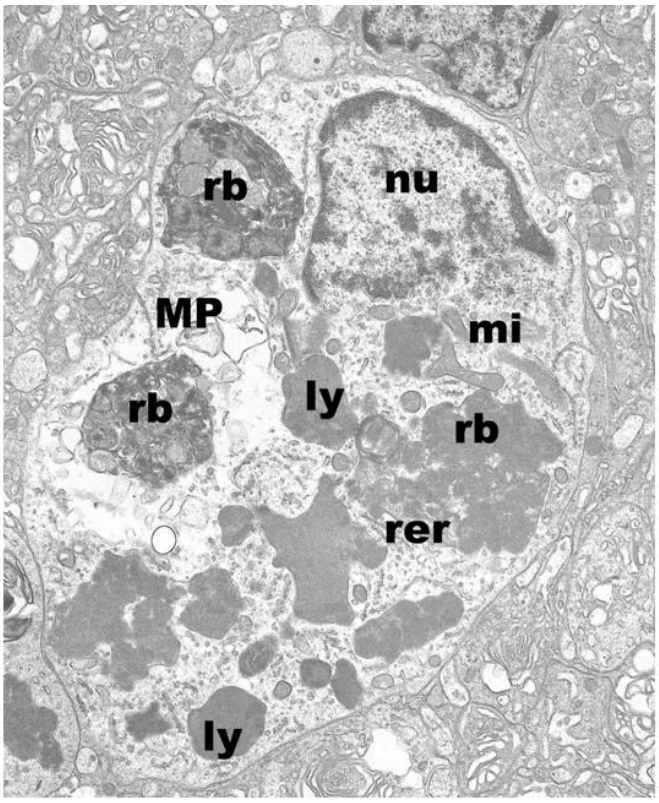


Fig. 43: Electron micrograph of an intraepithelial macrophage in adult bovine epididymal segment I. ly = lysosomes, mi = mitochondria, MP = macrophage, nu = nucleus, rb = residual body, rer = rough endoplasmic reticulum. (x 4400).

Intraepithelial macrophages (IEM) are occasionally encountered in the basal third of the epithelium. Their pseudopodia are insinuated between the high prismatic PC and the BC. Many mitochondria, a well developed Golgi apparatus and rER are found around a relatively

small heterochromatic nucleus (Fig. 43). Various profiles of lysosomes as well as dark globules, which may represent phagosomes, occur in the cytoplasm peripheral to the aforementioned organelles (Fig. 43).

4.2.4.2.2 Segment II

The height of the epithelium is uniform and the apical surface is equipped with long stereocilia. Abundance of numerous vacuoles and MVBs particularly in the apical cytoplasm are the most characteristic feature of PC in this segment (Fig. 44). Numerous round granules of variable electron density are seen between the Golgi zone and the nucleus and underneath the nucleus as well. Long cisternae of rER and some elongated mitochondria are observed peripheral to Golgi zone. Mitochondria are parallel to the longitudinal axis of the cell, especially close to the lateral cell membrane. Mitochondria are characterized by a comparatively dense matrix and transverse as well as tubular cristae. Small vesicles exist in between the mitochondria in the basal cytoplasmic zone.

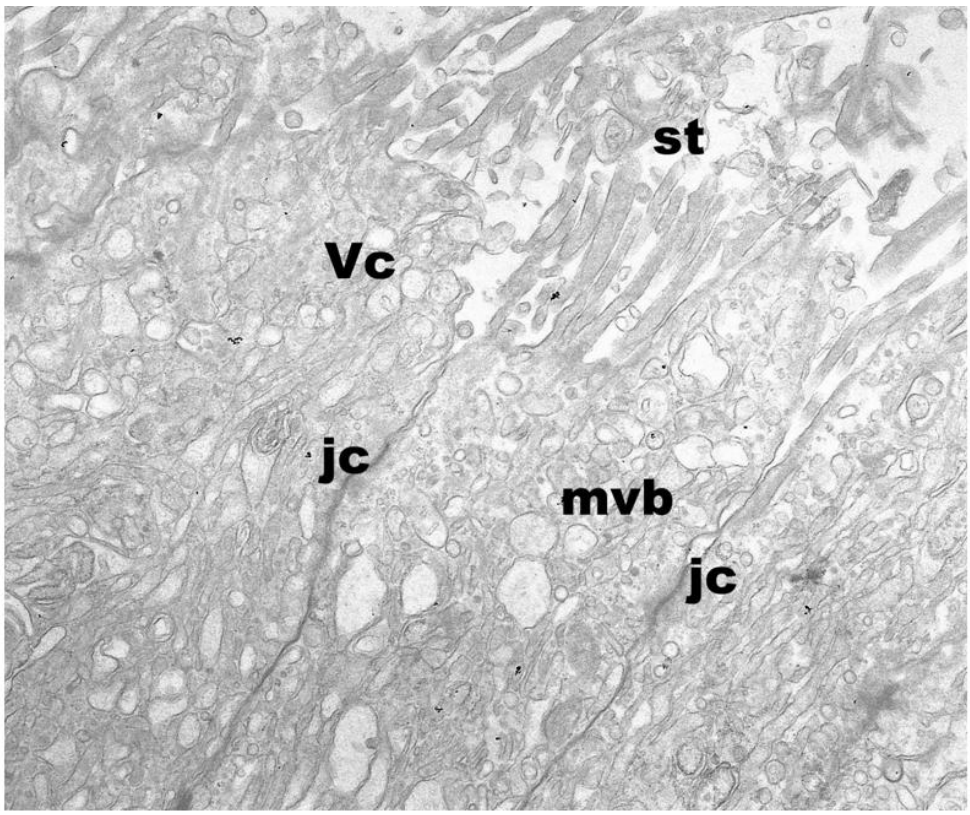


Fig. 44: Electron micrograph of apical region of the principal cells of adult bovine epididymal segment II. jc = junctional complex, mvb = multivesicular body, st = stereocilia, Vc = vacuole. (x 7000).

The BC are confined between the neighbouring PC and BL (Fig. 45). The contact of them with the BL is variable and depends on the extent of their contact area with the PC. They are characterized by paucity of organelles (Fig. 45). Lipid droplets are immediately located

adjacent to the nuclear membrane. There is a clear topographic proximity between lipid droplets and mitochondria. In addition to the four cell types present in segment I, a few numbers of AMRC exist in the epithelium of this segment. IEM appear in the basal zone of the epithelium. IEL are often encountered in immediate proximity to the subepithelial capillaries (Fig. 45). Their nuclei are round very heterochromatic and sometimes showed indentations. The cytoplasm is lighter and contains fewer organelles than that of the neighbouring cells. The Golgi apparatus is small and consists of some flat cisternae as well as small vesicles. Sporadic cisternae of rER, large number of ribosomes and occasionally dense bodies are found throughout the cytoplasm.

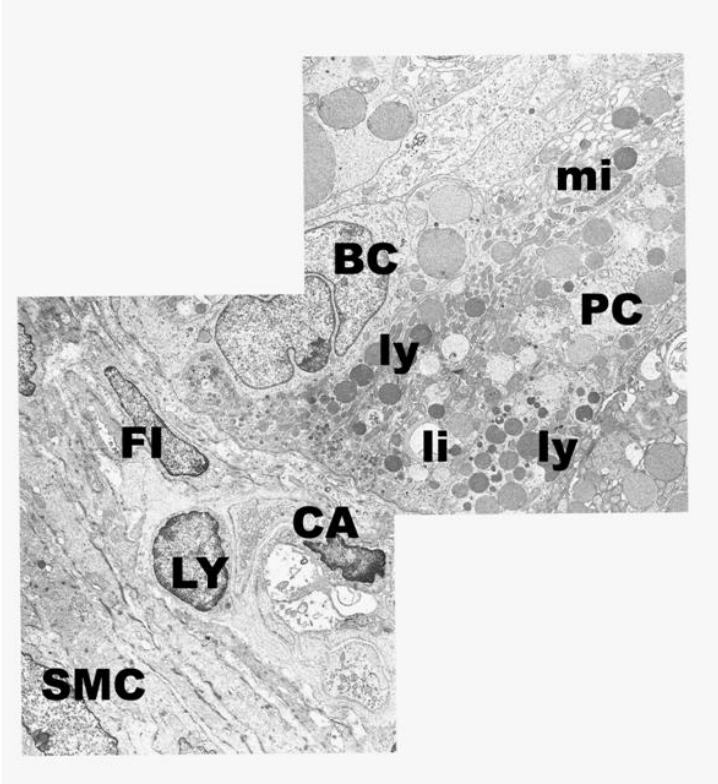


Fig. 45: Electron micrograph of the basal region of adult bovine epididymal segment II. BC = basal cell, CA = blood capillary, FI = fibroblast, li = lipid droplet, ly = lysosome, LY = lymphocyte, mi = mitochondria, PC = principal cell, SMC = smooth muscle cell. (x 7000).

4.2.4.2.3 Segment III

The apical surface of the PC presents several micropinocytotic invaginations, which are confined in between the stereocilia (Fig. 46). The cytoplasm of the apical zone contains numerous vesicles and short strands of the sparsely granulated ER (Fig. 46). Numerous microtubules originate from the base of the stereocilia. These microtubules are oriented in bundles parallel to the longitudinal axis of the cell. Numerous strands of rER as well as some mitochondria are found in the supranuclear cytoplasm.

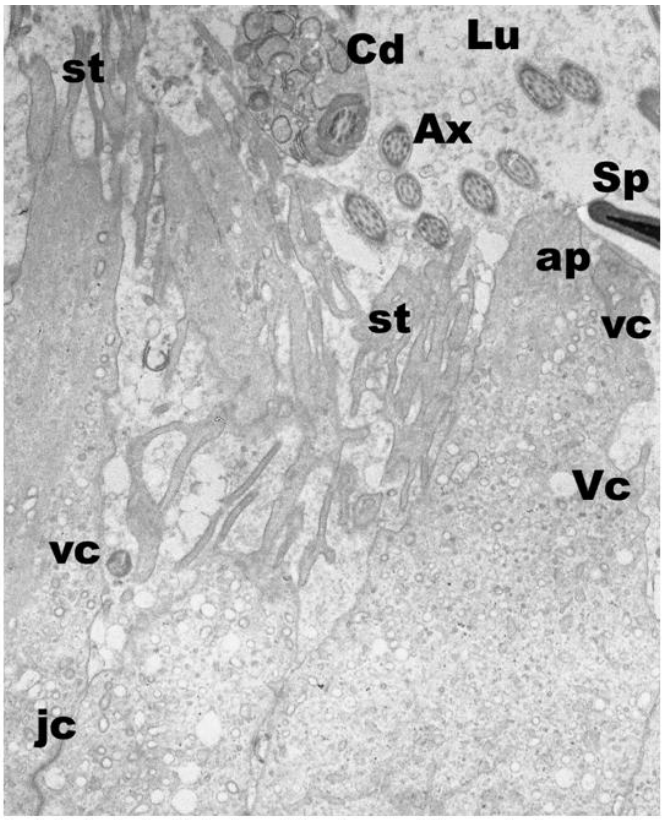


Fig. 46: Apical region of principal cells of adult bovine epididymal segment III. ap = apical protrusion, Ax = axoneme, Cd = cytoplasmic droplet, jc = junctional complex, Lu = lumen, Sp = sperm head, st = stereocilia, vc = vesicle, Vc = vacuole. (x 7000).

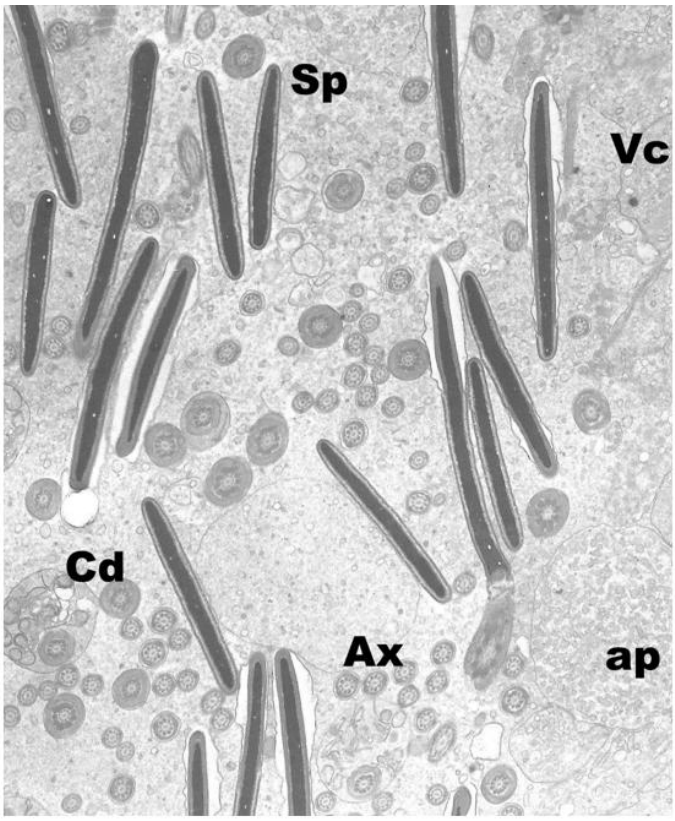


Fig. 47: electron micrograph of the lumen of adult bovine epididymal segment III. ap = apical protrusion of a principal cell, Ax = axoneme, Cd = cytoplasmic droplet, Sp = sperm head, Vc = vacuole. (x 3000).

The structure of the mitochondria is different from that of the previous two segments. They are characterized by numerous less electron dense crossly cut tubules, which are scattered in an electron dense matrix. In addition to the tubules, transverse cristae are also found. Golgi apparatus occupies a wide area above the nucleus and is surrounded laterally by mitochondria and rER. Immediate to the Golgi apparatus, there are variable forms of granules, mostly with a strong electron density. Similar granules are also found in the infranuclear area of the cytoplasm. The unusually irregular contour of the nucleus represents a characteristic feature of the PC in segment III. The nuclei are often subdivided into narrow chromatin strands, which bound together throughout narrow bridges. Many of the nuclei have basket-shaped nucleoli. The apical displacement of the nucleus makes the infranuclear zone of cytoplasm wider than that of segments I and II. BC presented a similar appearance and size as in the previous two segments, but the lipid droplets in the cytoplasm have been markedly increased (Fig. 48).

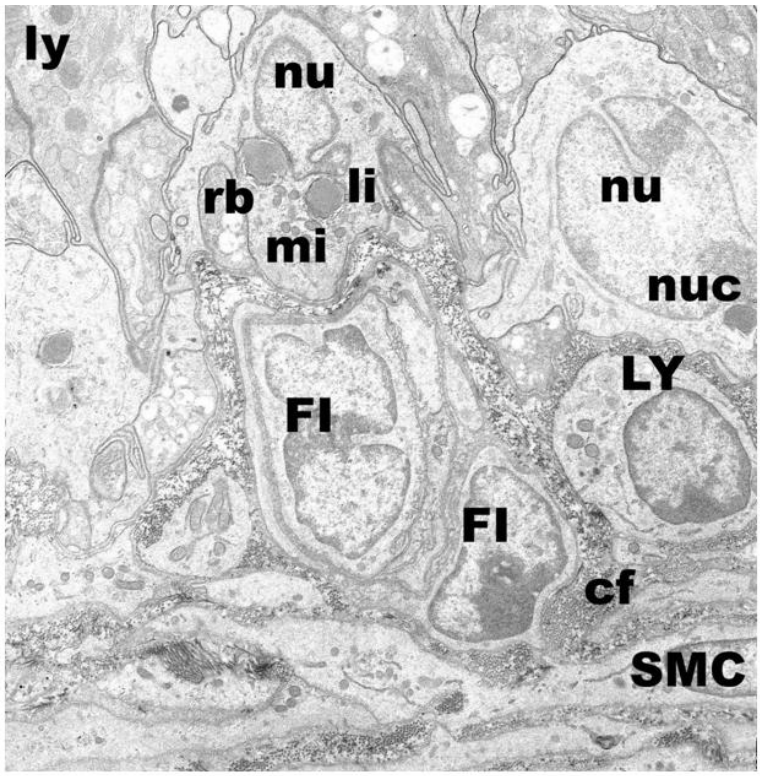


Fig. 48: Electron micrograph of the basal region of adult bovine epididymal segment III. cf = collagen fibrils, FI = fibroblast, li = lipid droplet, ly = lysosome, LY = lymphocyte, mi = mitochondria, nu = nucleus of basal cell, nuc = nucleolus of basal cell nucleus, SMC = smooth muscle cell. (x 3000).

AMRC are characterized by their round or oval nuclei which are found in the apical third of the epithelium (Fig. 49). Their light cytoplasm makes them easily identifiable from the denser PC. Mitochondria are the predominant organelles. They exist in a fair number and mainly apical to the nucleus. Apart from mitochondria, other organelles are less well-developed.

A varying number of vacuoles could sometimes be seen in apical cytoplasm (Fig. 49). The luminal surface of AMRC is provided by microvilli that are shorter than the stereocilia of the PC. A contact between them and the BL could not be proved.

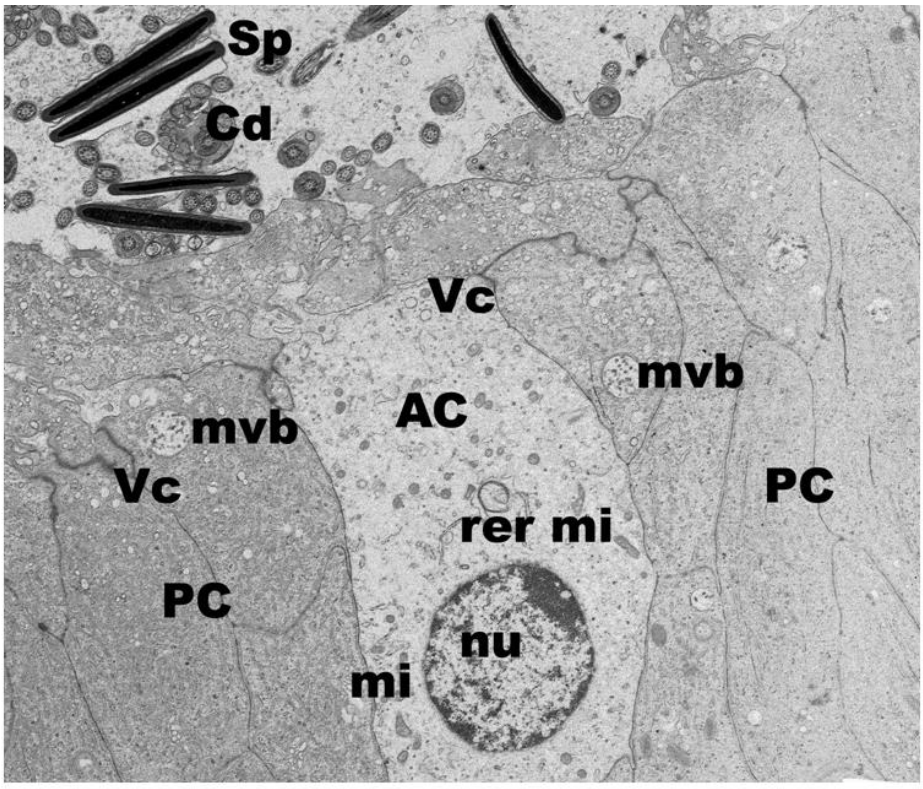


Fig.49: Apical region of the adult bovine epididymal segment III. AC = apical cell, Cd = cytoplasmic droplet, mi = mitochondria, mvb = multivesicular body, nu = nucleus, PC = principal cell, rer = rough endoplasmic reticulum, Sp = sperm head, Vc = vacuole. (x 3000).

The number of IEM is markedly increased and they are mainly found in the basal zones of the epithelium. The IEL (Fig. 48) exist in a similar number and appearance as in the previous two segments.

4.2.4.2.4 Segments IV and V

The cytological features of the PC are identical both in the fourth and the fifth segments. Thus both segments can be described together. The apical cytoplasm contains smooth and coated vesicles, subapical vacuoles and sometimes large MVBs. Bundles of microtubules are distributed among the vesicles and are parallel to the longitudinal axis of the cells. The size of the Golgi complex is comparatively smaller than that of the epididymal head segments (Fig. 50). The rER are located immediately above the nucleus. The nuclei are either elongated or oval and contain often one or more honeycomb-like nucleoli.

Mitochondria exist in a few numbers in the supranuclear area; however a great number of them are found in infranuclear and basal areas (Fig. 50). They are elongated and some of them

are twisted. Their cristae are lamellar and/or tubular. Mitochondrial matrix exhibits moderate electron density. In addition to mitochondria, the basal zone of the cytoplasm of PC contains a large number of small heteromorphic granules (Fig. 51, 52). BC exist in larger numbers in these epididymal segments than in those of the caput epididymis. They present morphological characteristics similar to those of the caput region. AMRC are found only rarely in both segments and present a similar morphology to those of the previous segments. Both IEM and IEL are found in lower numbers than in previous segments and exhibit the same cytological characteristics.

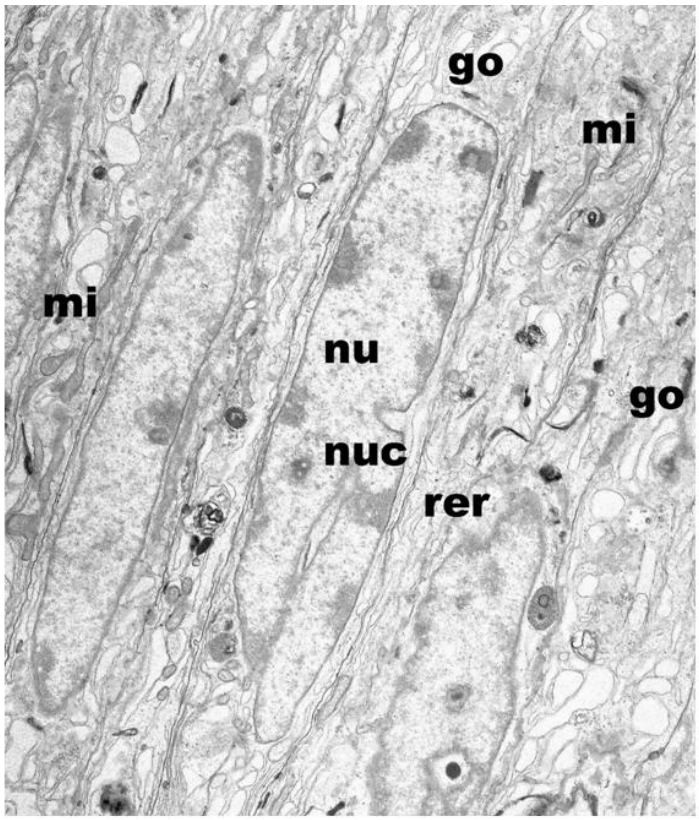


Fig. 50: Electron micrograph of principal cells of adult bovine epididymal segment IV. go = Golgi apparatus, mi = mitochondria, nu = nucleus, nuc = nucleolus, rer = rough endoplasmic reticulum. (x 3000).

4.2.4.2.5 Segment VI

The sixth segment corresponds to the cauda epididymidis. In addition to the components of the endocytotic apparatus in the apical cytoplasm, dense granules and vacuoles with or without central granule are distributed throughout the cytoplasm of the PC (Fig. 53).
The vacuoles with central granules contained myelin like material in the centre.

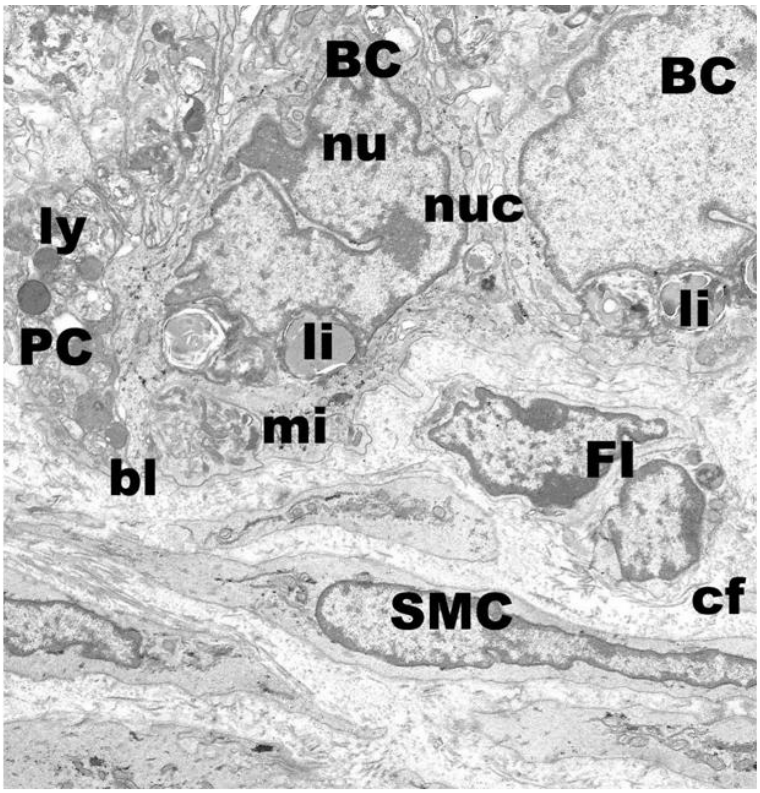


Fig. 51: Electron micrograph of the basal area of adult bovine epididymal segment IV. BC = basal cell, bl = basal lamina, cf = collagen fibrils, Fl = fibroblast, li = lipid droplet, ly = lysosome, nu = nucleus, nuc = nucleolus, PC = principal cell, SMC = smooth muscle cell. (x 3000).

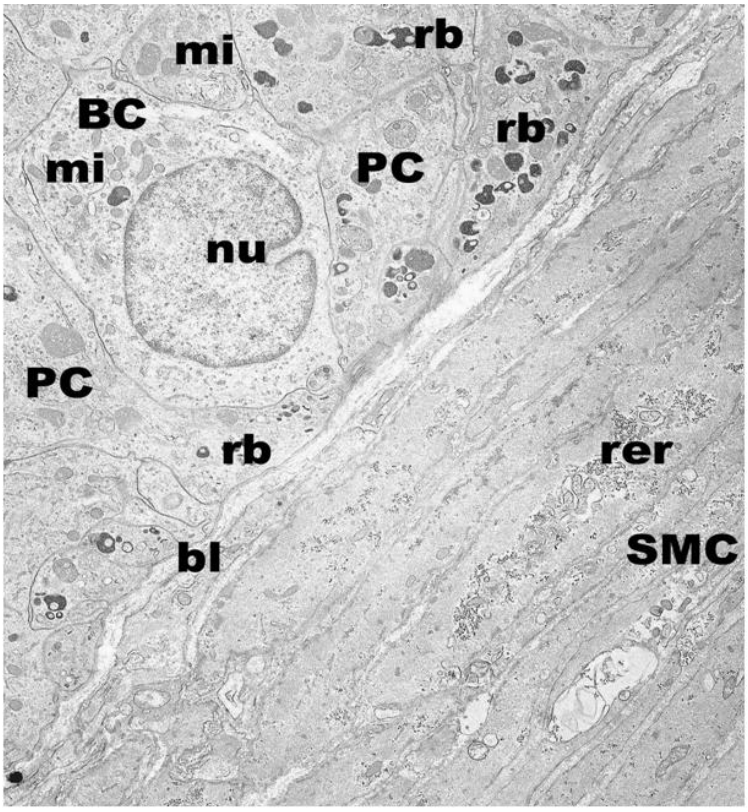


Fig. 52: Electron micrograph of the basal region of the adult bovine epididymal segment V. BC = basal cell, mi = mitochondria, nu = nucleus, PC = principal cell, rb = residual body, rer = rough endoplasmic reticulum, SMC = smooth muscle cell. (x 3000).

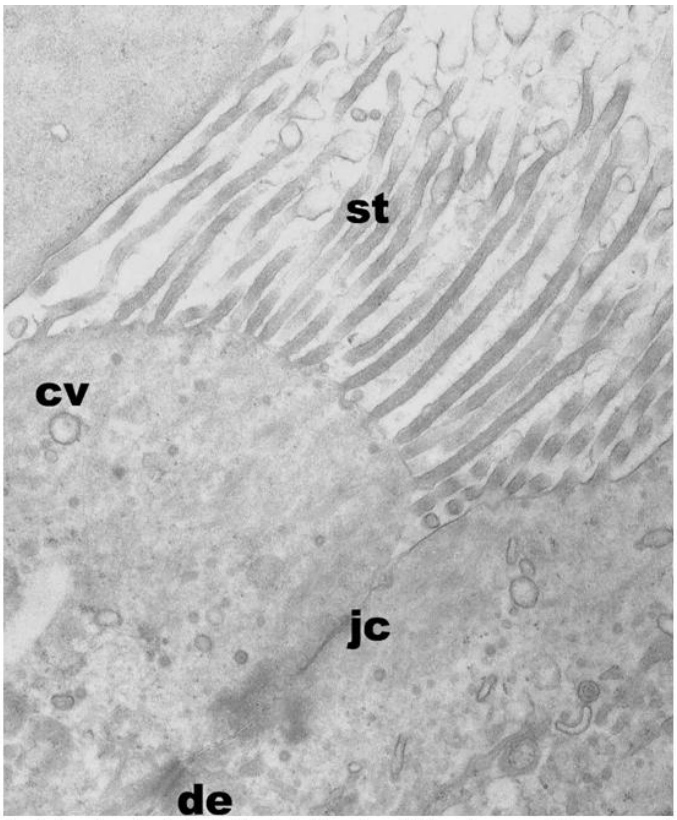


Fig. 53: Electron micrograph of apical region of principal cells of adult bovine epididymal segment VI. cv = coated vesicle, de = desmosome, jc = junctional complex, st = stereocilia. (x 12,000).

They are non membrane-bound and are surrounded by many mitochondria. The supranuclear cytoplasm possesses a characteristic stratification of the cellular organelles. In the uppermost zone of supranuclear cytoplasmic area, the number of vesicles is decreased in comparison to those of the previous segments. Free ribosomes occur in a vast number and frequently in the form of polyribosomes. The second zone of the supranuclear cytoplasmic area is wide and is characterized by many short strands of rER. The third zone is occupied with Golgi complex. The latter consists of many stacks of cisternae as well as numerous vesicles at the periphery of them. The nuclei of PC possess a very irregular contour. They are similar to those of the third segment. The nuclear material is often subdivided into many longitudinally oriented chromatin fragments that are joined together through bridges (Fig. 55). The nucleoli are found at the periphery of the nuclei. Electron-dense lysosomal granules are found near to the nucleus (Fig. 55). They exhibit variable sizes and different forms. Different from the proximal segments, a large number of mitochondria appear around the nuclei (Fig. 55). Furthermore, mitochondria are also found in the infranuclear and basal areas. Mitochondria are very heteromorphic. BC are similar to that of the proximal segments in their size and forms (Fig. 55). AMRC are not found in the cauda epididymidis. IEM and IEL occur in variable numbers in the neighbouring tubules of the same section. They present cytological characteristics similar to those of the proximal segments.

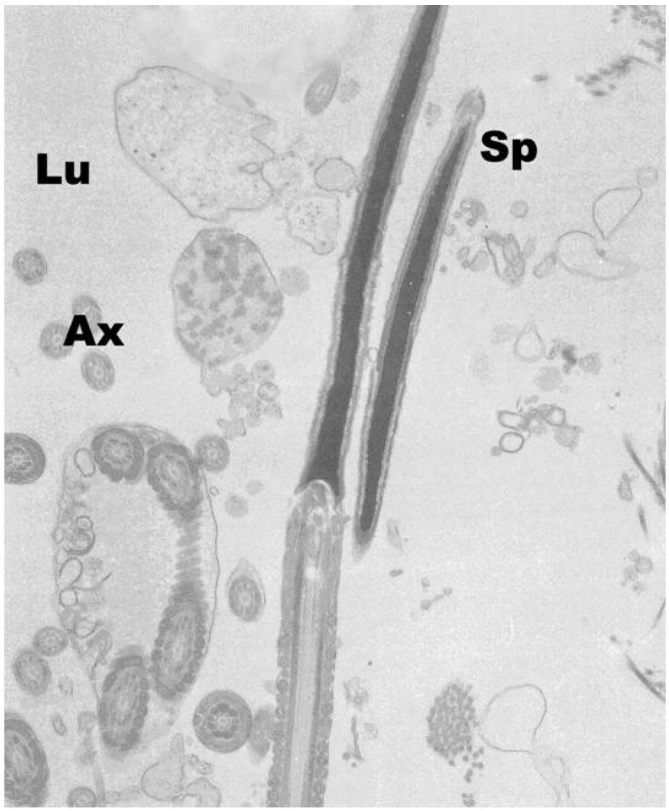


Fig. 54: Electron micrograph showing sperm in the lumen of epididymal segment VI. Ax = axoneme, Lu = lumen, Sp = sperm. Note absence of cytoplasmic droplet. (x 3000).

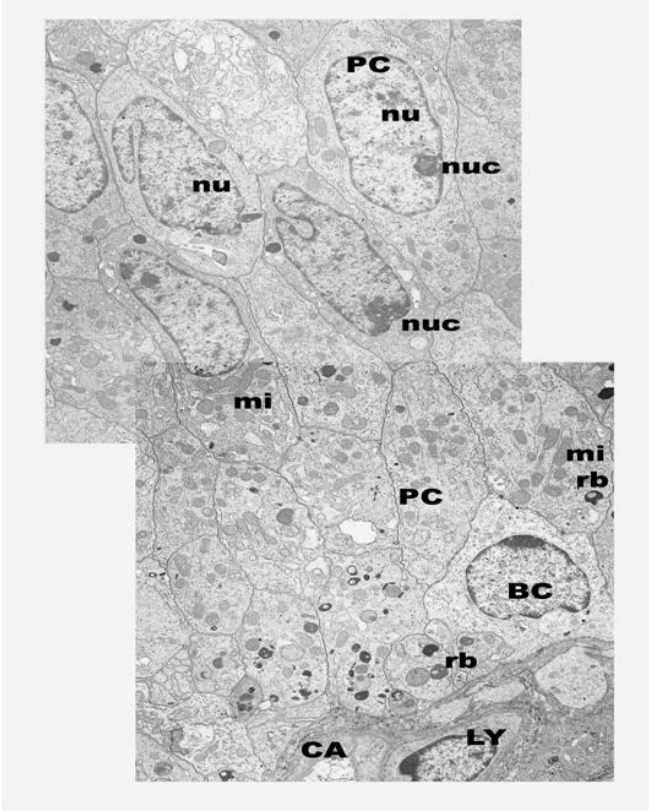


Fig. 55: Electron micrograph adult bovine epididymal segment VI. BC = basal cell, CA = blood capillary, ly = lysosomes, LY = lymphocyte, nu = nucleus, nuc= nucleolus, PC = principal cell, rb = residual body. (x 3000).

4.2.4.3 Extraepithelial, peritubular and interstitial structures

The basal lamina (BL) of the epididymal epithelium is distinctly developed along the length of the epididymal duct (Fig. 51, 52). A large number of hemidesmosomes exist between the BC and the BL. The subepithelial capillaries are still always separated from the epithelium through the BL. Peripheral to the BL, a thin layer of CT consisting of fibroblasts and collagen fibrils is longitudinally extended (Fig. 45, 48, 51). A definite line of demarcation between the collagen fibrils and the SMC is not uncommon.

Cytological characteristics of SMC show minimal variation between the different segments of the epididymal duct. The SMC in the caput and the corpus regions have a spindle shaped contour, whereas that of the cauda epididymidis possess truncated ends. Definite basal laminae surround these cells and additionally each cell is separated from the neighbouring cells through a layer of collagenous fibrils. The elongated nuclei of SMC adapt themselves completely to the cell bodies leaving a narrow peripheral cytoplasmic rim. The predominant component of the cytoplasm is formed by bundles of myofilaments, which originate from thickening of the cell membrane and run obliquely or parallel to the longitudinal axis of the cell. Micropinocytotic vesicles are seen at the sites of the cell membrane, which are devoid of the membrane thickenings that give origins for the myofilaments. The mitochondria are of the cristae type and have elongated oval shape. They exist in a large number and occur mainly at the poles of the nucleus. The muscle cells have a moderately developed Golgi apparatus as well as some strands of rER. Many glycogen granules are found throughout the cytoplasm.

Interstitial spaces are occupied by loose CT incorporated blood and lymph vessels as well as nerve fibres. The collagen fibres of the interstitium are loose and randomly arranged. The cellular elements of the CT include fibroblast, macrophages, granulocytes, lymphocytes and mast cells.

5. Discussion

5.1 Light histological structure of bovine epididymis

5.1.1 Prenatal development

When replaced by a more efficient excretory system (formation of the metanephros), mesonephros regresses in a craniocaudal direction leaving the most caudal portion of the mesonephric duct and the tubules associated with it to be incorporated into the male genital system (Arey, 1965; Günther, 1995; Dyce et al., 1996; Moustafa and Hafez, 1971). Regression of the mesonephros in the bovine foetus begins after 49 pcd and the mesonephric tubules are then converted into efferent ductules (ED) between 60 and 150 pcd (Moustafa and Hafez, 1971; Rüsse and Sinowatz, 1991). Although the events of development of the ED appears at times near from that given by others, Wrobel (2001) and Wrobel and Schimmel (2001) assumed that the bovine ED are not formed by transformation of primary mesonephric tubules, but as a new set of secondary mesonephric tubules which originate as outgrowths from the dorsal wall of the mesonephric giant corpuscle (MGC) at about 47 pcd. Between 40 and 45 pcd the rete testis anlage is a nearly exclusively extratesticular structure situated between the cranial pole of the testis and the ventromedial aspect of the MGC. The extratesticular rete blastema proliferates and then bypasses the MGC and mesonephric duct to join the emerging ED at 50-60 pcd (Wrobel, 2001). Furthermore, establishment of the urogenital junction takes place at about 85 pcd, when the growing ED reach the mesonephric duct and the rete channels possess patent lumina. Our results are in accord with that of Wrobel (2001) in this regard. The proliferating mesenchymal cells surrounding the epithelium of the ED are arranged in several concentric layers. These mesenchymal cells are the precursors of the periductular musculature (Wrobel, 2001; Wrobel and Schimmel, 2001).

The bovine ED lengthen and strongly coil at the fourth month of gestation (110 pcd, 24 cm CRL) forming the coni vasculosi in the caput epididymidis (Rüsse and Sinowatz, 1991). The bovine efferent ductular epithelium displays two periods of high cytological differentiation, first is a prenatal when the foetal Leydig cell population is well developed and second is a postnatal due to the pubertal rise in androgens (Wrobel, 2001; Wrobel and Schimmel, 2001). Similarly, Rüsse and Sinowatz (1991) reported that nests of foetal Leydig cells are abundant in the bovine foetal testis during the differentiation of the male excurrent ducts (3 to 4 months of gestation). Moreover, the epithelium in the mesonephros-derived tissues including rete

testis, ED and epididymis possess a higher capacity to express androgen receptors than the rest of the testicular tissues (You and Sar, 1998).

The epididymal duct develops from the portion of the mesonephric duct where the caudal ED open (Rüsse and Sinowatz, 1991). After degeneration of the mesonephros (at about 49 pcd), the mesonephric duct gives rise to epididymis and deferent duct between 60 and 150 pcd (Moustafa and Hafez, 1971; Rüsse and Sinowatz, 1991). At about 110 pcd (4^{th} month of gestation) and a 24 cm CRL, the epididymal duct lengthens and begins to convolute strongly (Rüsse and Sinowatz, 1991). The convoluted epididymal duct forms the caput, corpus and cauda epididymidis which continues as ductus deferens (Moustafa and Hafez, 1971; Rüsse and Sinowatz, 1991). The findings of the present work are consistent with the aforementioned data. Similar findings are also recorded in human foetal epididymal duct (Krutsiak and Kumka, 1988). The epithelium lining the epididymal duct is simple cuboidal to low columnar at the beginning and differentiates later to the characteristic slender, tall columnar cells with stereocilia (Moustafa and Hafez, 1971; Rüsse and Sinowatz, 1991). In human foetal epididymis, the epithelium possesses two cell types: tall columnar cells and short pyramidal basal cells (Gould and Bernstein, 1979). Contrary to the condition in human, the epididymal epithelium in newborn calf is simple columnar in all regions with the exception of the distal portion of the cauda (Abd El-Raouf, 1960). My findings support those of Abd El-Raouf (1960), since the epithelium lining the entire length of the epididymal duct was simple columnar in the full-term calf (285 pcd, 90 cm CRL). The differentiation from simple columnar to pseudostratified epithelium is due to the appearance of the basal cells, which are probably developed from the columnar cells (Abd El-Raouf, 1960). This differentiation was found to be completed in all different regions of the duct at about 32 weeks postnatal.

The epithelium of the human foetal epididymis possesses secretory activity as indicated by diastase-resistant, PAS-positive secretions (Zondek and Zondek, 1980). Sometimes PAS-positive granules are found in the cytoplasm of the cells bordering the lumen. This secretory activity may be ascribed to the foetal androgens (Zondek and Zondek, 1980). Similar findings are found in the caput region of bovine foetal epididymis (Moustafa and Hafez, 1971) as indicated by secretory granules and droplets in the apical cytoplasm of the epithelium at 150 pcd. The occurrence of this secretory activity is possibly concomitant with the proliferation of the foetal Leydig cells (Rüsse and Sinowatz, 1991). It is noteworthy that the epithelium of all mesonephros-derived tissues including epididymis expresses more androgen receptors compared to the germinal epithelium (You and Sar, 1998). Furthermore, modifications of the apical cytoplasm of the mesonephric duct in the rat foetus (Flickinger, 1969) include increases

in the size and number of microvilli, the formation of numerous pits in the plasma membrane between the microvilli and the appearance of many coated vesicles. These modifications have been associated with absorptive activity in the adult epididymis (Flickinger, 1969). Similarly, Wrobel (2001) reported that the epithelial cells lining the bovine ED undergo cytological differentiation on about the 3rd month of gestation. The differentiation proceeds in a proximodistal direction. Consequently the columnar epithelium of the proximal portions is already differentiated into ciliated and reabsorptive nonciliated cells. The latter showed a brush-border and endocytotic apparatus. These changes coincide with proliferation of the foetal Leydig cells (Rüsse and Sinowatz, 1991) and are suggested to be in response to an increase in androgen. Furthermore, similar findings were recorded in foetus of rhesus monkey (Alexander, 1981). The author reported that the cuboidal epithelial cells lining the foetal epididymal duct undergo androgen-dependent cytodifferentiation.

The epididymal epithelium is girdled by a prominent basement membrane (Moustafa and Hafez, 1971). The periductal accumulation of basement membrane material may reflect an incipient epididymal differentiation (Paranko et al., 1985). In agreement with Moustafa and Hafez (1971) and Rüsse and Sinowatz (1991), the foetal epididymal epithelium was surrounded by a thin layer of smooth muscle cells and the thickness of this layer increases distalwards.

5.1.2 Adult bulls

It is noteworthy that I cite that the functional correlates are described in a separate section (see 5.5) at the end of the discussion. However, in some items, functions may be briefly discussed.

5.1.2.1 Light histological characteristics of efferent ductules

Efferent ductules (ED) are the part of the male excurrent ducts, which connect the rete testis with the epididymal duct (Patt and Patt, 1969; Ilio and Hess, 1994; Wrobel, 1998). Their numbers range from 4 to 8 in laboratory animals including rat, mouse, hamster, and guinea pig (Ilio and Hess, 1994) and from 12 to 23 in large animals including bull, ram, goat, stallion, boar and dog (Hemeida et al., 1978). The epithelial lining of the ED, depending on the species and the author's view point, is classified either simple or pseudostratified columnar, composed of ciliated cells, nonciliated cells, and a few basal cells as well as intraepithelial lymphocytes and macrophages (Ilio and Hess, 1994). For example, Tröger (1969) mentioned that the bovine ED are lined by a pseudostratified columnar epithelium composed of ciliated cells, principal (nonciliated) cells and scattered small basal cells.

Contrary to Tröger (1969) who mentioned that the ciliated cells carry stereocilia, Wrobel (1972) recorded that they are equipped with kinocilia and some microvilli scattered in-between. Furthermore, Goyal and Hrudka (1981) reported that the epithelium of bovine ED consists of ciliated and nonciliated cells and a smaller number of intraepithelial lymphocytes and macrophages. They, however, do not refer to the existence or absence of basal cells described by Tröger (1969) and Wrobel (1972). The caprine ED are lined by a pseudostratified columnar epithelium composed of ciliated, nonciliated and basal cells (Gray et al., 1983). However, Goyal and co-workers (1992) described the epithelium of caprine ED as simple columnar with ciliated and nonciliated cells and do not describe the occurrence of basal cells. Although the epithelium of hamster (Flickinger et al., 1978) and guinea pig (Hoffer and Greenberg, 1978) ED is made up of two cell types (ciliated and nonciliated cells) the former was described to be pseudostratified and the latter to be simple columnar. The equine ED are lined by a pseudostratified columnar epithelium consisting of nonciliated, ciliated and basal cells as well as migrating leucocytes (López et al., 1989). However, Arrighi and co-workers (1993) mentioned that ED of stallion are lined by epithelium consisting of ciliated and nonciliated cells as well as some migrating lymphocytes. From the aforementioned data in different species, it is obvious that there is discrepancy in the classification of the epithelium among different species and even within the same species when studied by different authors.

The present work showed that the epithelium of the bovine ED is simple columnar with ciliated cells. Similar findings were recorded in the same species by Goyal and Hrudka (1981). In agreement with Wrobel (1972) and with Goyal and Hrudka (1981), ciliated cells in the epithelium of bovine ED are easily distinguishable from nonciliated cells, not only by their cilia but also by the position of their heterochromatic nuclei near the luminal end of cells. A similar morphology is exhibited by ciliated cells in other species including hamster (Flickinger et al., 1978), goats (Gray et al., 1983; Goyal and Williams, 1988; Goyal et al., 1992) and stallions (López et al., 1989). However, in guinea pig (Hoffer and Greenberg, 1978) and in monkey (Ramos and Dym, 1977 b), the nuclei of the ciliated cells are mostly euchromatic.

Whereas ciliated cells possess a similar morphology in mammalian species studied, non-ciliated cells present variable morphological characteristics in the different species.

In laboratory animals including rat (Hamilton, 1975), guinea pig (Hoffer and Greenberg, 1978) and hamster (Flickinger et al., 1978), they present a single cell type which is described as absorptive cells. In other species they have been classified into three (men, Morita, 1966;

bulls, Goyal and Hrudka, 1981; goats, Gray et al., 1983; Goyal and Williams, 1988; Goyal et al., 1992) or even four cell types (dogs, Hess and Bassily, 1988).

My findings revealed that, on the basis of their cytological characteristics, nonciliated cells of bovine ED can be categorized into three cell subtypes. The first type contained neither granules nor vacuoles, the second type displayed plenty of diastase-resistant, PAS-positive granules in the cytoplasm and the third type possessed a cytoplasm abundant in vacuoles. The luminal surface of all three cell types possessed microvilli, apical protrusions or both of them. Apical protrusions may contain granules or vacuoles and may be found released in the lumen, along with granules or vacuoles. Goyal and Hrudka (1981) reported similar findings in the same species. On the other hand, nonciliated cells in the bovine ED were early described as principal cells by Wrobel (1972), who assumed that nonciliated (principal) cells represent one cell type which exists in two functional stages of the absorptive process. Goyal and Hrudka (1981) criticized the assumption of the so-called one-cell concept reflected by the functional state of the same cell described by Benoit (1926) and Wrobel (1972). Benoit (1926) described three types of nonciliated cells in bovine ED, which in his opinion represent only different secretory phases of the same cell. On the other hand, Wrobel (1972) interpreted the variation between nonciliated cells as two phases of the absorptive process. He mentioned that the vacuoles are a result of fluid absorption, which then condense into granules and are then eventually released into the lumen through apical protrusions. Goyal and Hrudka (1981) disagree with this theory and assume that it does not conform to their findings for many reasons, which include:

- Type II cells exist in proximal but not in distal segment, and vice versa, type III cells exist in distal but not in proximal segment.
- Vacuoles do not convert to granules and vice versa.
- The contents of the granules and vacuoles differ in histochemical and physical properties.
- The first appearance of granules in histogenesis precedes that of vacuoles by several weeks.

Goyal and Hrudka (1981) therefore postulate that nonciliated cells of the bovine ED include at least two cellular subtypes, type II cells inhabiting the proximal segment and type III cells residing in distal segment of the ED. Both subtypes presumably originate from type I cells, which seem to be the inactive form of both categories and are found in both segments of ED. Furthermore, the transition zone between the proximal and distal segment presents rare transitional cells showing granules and vacuoles concomitantly (Goyal and Hrudka, 1981).

My findings are consistent with those of Goyal and Hrudka (1981) who assumed that nonciliated cells possess two kinds of secretory activity and mentioned that type II (granular) cells in proximal portions of bovine ED engage in the formation of specific granules and type III (vacuolar) cells in distal portion elaborate specific vacuoles. The same authors stated that all nonciliated cells are provided with resorptive apparatus and microvilli, apical protrusions or both of them. They also recorded that apical protrusions may contain granules or vacuoles. It becomes evident from the aforementioned data that all nonciliated cells perform both absorptive and secretory activities (Goyal and Hrudka, 1981). Similarly, nonciliated cells of caprine ED are classified into three categories, type I, II and III cells (Goyal and Williams, 1988; Goyal et al., 1992). The authors mentioned that these cells are not randomly distributed within the epithelium, but gradually differentiate from type I to type II to type III cells along the length of each ED.

Contrary to Nicander (1970) and Hamilton (1972), who stated that apical protrusions in the epithelium of ED are artefacts as a consequence of poor or delayed fixation and mechanical damage, Wrobel (1972) recorded that apical protrusions in the bovine ED are distinctly distinguishable from artefacts. Similarly, Goyal and Hrudka (1981) disproved the role of fixation as a cause of incidence of apical protrusions, through the use of different fixation techniques. These protrusions are found after the use of different methods of fixation and even in unfixed (frozen) sections (Wrobel, 1972). On the other hand, Benoit (1926) claimed that the incidence of apical protrusions (blebs) in bovine ED could be prevented by perfusion fixation. Furthermore, apical protrusions are recorded in human ED (Morita, 1966; Holstein, 1969) and may account for their secretory activity.

The bovine ductular wall is supported by a thin lamina propria and a thin layer of smooth muscle cells and their lumina are empty or contain few spermatozoa. The thickness and the number of the smooth muscle cells increase towards the epididymis. Moreover, the peritubular interstitial connective tissue (CT) is very rich in CT fibres and contains relatively frequent mast cells which are strongly stained with Alcian blue. Similar findings were reported in the same species (Wrobel, 1972).

5.1.2.2 Light histological characteristics of epididymis

The traditional macroscopic segmentation of the epididymis into head, body and tail is insufficient to reflect the structural-functional relationship of this organ (Glover and Nicander, 1971). Based on histological, histochemical and ultrastructural characteristics, the epididymis of most mammalian species can be subdivided into several segments. The number and

distribution of these segments is species-specific (Wrobel, 1998). The number of epididymal segments is three in dog (Chandler et al., 1981; Ruhl, 2001) and camel (Tingari and Moniem, 1979), four in rat (Hamilton, 1975) and cat (Sanchez, 1998), five in hamster (Flickinger et al., 1978), mouse (Takano, 1980) and goat (Goyal and Williams, 1991), six in bull, ram and stallion (Nicander, 1958), llama (Delhon and Lawzewitsch, 1994), buffalo-bull (Goyal and Dhingra, 1975) and boar (Wrobel and Fallenbacher, 1974), seven in guinea pig (Hoffer and Greenberg, 1978) and eight in rabbit (Nicander, 1957), buffalo-bull (Abdou et al., 1985) and man (Holstein, 1969). Furthermore, Glover and Nicander (1971) stated that the number of epididymal segments may vary within the same species depending upon the criteria of classification.

On the basis of histological and histochemical characteristics, the bovine epididymis was subdivided into six segments (Nicander, 1958; Erkmann, 1971; Sinowatz, 1981). These characteristics include the regional variation of epithelial height, the size, the position and the form of the nuclei of the PC, the existence of certain cell types (principal, basal and apical cells), and the luminal diameter (Nicander, 1958). Histochemical (Erkmann, 1971; Sinowatz, 1981) and ultrastructural (Sinowatz, 1981) studies confirmed this system of segmentation.

The results of the present work are similar to those of Nicander (1958). On the other hand, Goyal (1985) recorded similarities in the epithelial height, luminal diameter and smooth muscle wall thickness among regions II-IV and suggested that these regions may be classified under one region. However, Erkmann (1971) and Sinowatz (1981) stated that both of the fourth and fifth segments show close similarities and suggested that they may form together one region (corpus epididymidis). Both authors assumed that the subdivision of the corpus epididymidis into fourth and fifth segments is unnecessary. The findings of the present work are in agreement with those of Erkmann (1971) and Sinowatz (1981).

The mean value of the luminal diameter of the epididymal duct reached 260.80 ± 32.47, 272.80 ± 26.47, 260.60 ± 22.17, 227.40 ± 08.35, 220.60 ± 12.12 and 429.20 ± 41.72 µm in the epididymal segments I through VI respectively. However, Goyal (1985) showed that the luminal diameter of the bovine epididymal duct reached 146, 178, 171, 171, 104 and 246 µm in the segment I through VI respectively. Since the present work was carried out on Holstein bulls and that of Goyal (1985) involved Hereford bulls, the difference in the luminal diameter could be ascribed to breed variation. Furthermore, the lumina of the first segment were frequently irregular and sometimes are stellate-shaped. They contain small numbers of spermatozoa. This was also reported by Nicander (1958), Sinowatz (1981) and Goyal (1985). The lumen of the second segment is regular, oval or even round. Similar findings were given

by Nicander (1958) and Erkmann (1971). The lumina of the second segment contain small (Erkmann, 1971) or moderate (Nicander, 1958) amount of spermatozoa. The lumina of the third segment are regular round or slightly oval and crowded with spermatozoa (Nicander, 1958; Erkmann, 1971; Sinowatz, 1981) as well as many cells with round nuclei, which presumably are germinal cells (Sinowatz, 1981). The lumina of most tubules in the segment IV present regular round outlines. Furthermore, the lumina were filled with spermatozoa (Nicander, 1958; Sinowatz, 1981; Goyal, 1985). The lumina of the tubules of the fifth segment are narrower than those of the fourth one. Moreover, the lumina of this segment are filled with numerous spermatozoa (Sinowatz, 1981; Goyal, 1985). The lumina of the tubules of the sixth segment are very wide (429.20 ± 41.72 μm) and are surrounded by a somewhat regular epithelium. However, Sinowatz (1981) mentioned that the proximal portion of the bovine epididymal tail showed slightly folded epithelium and that the folding faded downwards in the direction of ductus deferens. My findings are consistent with these results. In the sixth segment the luminal diameter reaches its maximum. It contains the highest sperm cell concentration. Similar findings were reported also for other species (ram, Nicander, 1958; goat, Goyal and Williams, 1991; boar, Wrobel and Fallenbacher, 1974); stallion, Nicander, 1958; cat, Sanchez et al., 1998; dog, Chandler et al., 1981; Ruhl, 2001; camel, Tingari and Moniem, 1979; llama, Delhon and Lawzewitsch, 1994; buffalo-bull, Abdou et al., 1985; human, Holstein, 1969). This clearly demonstrates that the main function of the sixth segment is sperm storage.

The mean value of the epithelial height was 105.39 ± 6.81, 77.01 ± 4.19, 75.99 ± 14, 81 ± 2.64, 92.79 ± 4.59 and 53.60 ± 4.22 μm in the epididymal segment I through VI. The data for epithelial height in the various segments of the epididymal duct agreed mostly with data given in previous studies (Sinowatz, 1981; Goyal, 1985). The epithelial height of the epididymal duct of the first segment was variable (72-156 μm). This results in an irregular contour (festooned outline) which may cause stellate-shaped lumina. Similar to the first segment the epithelium of the fifth segment has variable height (60-144 μm). This causes the star-shaped lumina of this segment. The epithelium height in the sixth segment was lower than in any other segment of the epididymis. Similar findings were found by Erkmann (1971), Sinowatz (1981) and Goyal (1985) in the same species. The epithelium lining the ductus epididymidis is pseudostratified columnar. It contains mainly four cell types, namely: principal cells (PC), basal cells (BC), intraepithelial lymphocytes and macrophages. Apical mitochondria-rich cells (AMRC) represent another cell type, which exist in certain epididymal segments. PC are the major cell type in the epididymal epithelium. These findings agree well with previous studies

(Erkmann, 1971; Sinowatz, 1981; Goyal, 1985).

The luminal surfaces of the PC are equipped with stereocilia. As found in previous studies (Nicander, 1958; Sinowatz, 1981) the height of the stereocilia decreased from 21.99 ± 0.79 µm in the first segment (caput epididymidis) to 7.20 ± 0.47 µm in the sixth segment (cauda epididymidis). This may reflect their important role in the resorptive activity. Most of the rete testis fluid is reabsorbed in the caput region (Carbo, 1965). It is noteworthy that the stereocilia of the first segment showed a distinct staining with Alcian blue. Similar findings were reported by López and co-workers (1989) in the equine caput epididymidis. They ascribed this staining pattern to carboxyl group of glycoproteins and/or glycolipids associated to sialic acids or sulphate polysaccharides in the stereocilia of this segment.

In agreement with previous studies (Nicander, 1958; Erkmann, 1971; Sinowatz, 1981; Goyal, 1985) the nuclei of the PC in the different segments displayed distinct variations. These include their shape, location and length. The nuclei of the PC in the first segment are placed near the basal membrane. They are elongated oval. Their length measures 17.32 ± 0.99 µm and they possess two to three distinct nucleoli. A few scattered nuclei are found near the apical area of the epithelium. Some of them show mitotic activity. Mitoses in the apical area of the epididymal duct were also recorded by Nicander (1958), Sinowatz (1981) and Goyal (1985). The nuclei of the PC in the second segment are short ovoid or even round. They measure 11.74 ± 1.11 µm. They are arranged in a multilayered nuclear zone in the basal area of the epithelium without a line of demarcation between them and those of the BC. The nuclei of the PC in segment III of the epididymal head are elongated with slightly irregular outlines. They measure 22.72 ± 0.68 µm and they are located at a higher level in the cytoplasm. This creates a wider zone of infranuclear cytoplasm. The nuclei of the PC of the fourth and fifth segment are elongated with irregular outlines. They are somewhat darker than those of the head region. Furthermore, they are found at different levels of the epithelium; however the most basal nuclei left a moderately wide infranuclear cytoplasmic zone so that the nuclei of the BC are clearly identifiable. They measure 23.52 ± 0.97µm and 25.92 ± 1.24 µm in the fourth and fifth segment respectively. The nuclei of the PC in the sixth segment possess irregular outlines and are deeply invaginated. They are highly basophilic, elongated and measure 21.48 ± 1.13 µm.

The BC present a similar morphological characteristics along the length of the epididymal duct. The BC in the first two segments have pyramidal to triangular nuclei, which are located in the centre of the cell and are mainly surrounded by a narrow streak of cytoplasm. They are located on the basal membrane and characterized by a high nuclear: cytoplasmic ratio. They

are found only sparsely in the first two segments of the bovine epididymis. Similar findings were recorded by Nicander (1958); Erkmann (1971); Sinowatz (1981) in the same species and by Nicander (1958) in corresponding regions of ovine epididymis. In the third segment the number and size of BC increases compared to those of the previous two segments. Sinowatz (1981) recorded that the BC in this segment present a similar appearance and size as in the former two segments. In the equine epididymis, Nicander (1958) found that the BC are smaller in segment III than in the previous two segments of the head. The BC in the fourth segment are somewhat more numerous than in the head region. Similar results were found by Nicander (1958); Erkmann (1971); Sinowatz (1981) in the bull, and by Nicander (1958) in the equine and ovine epididymis. In accordance with previous studies (Nicander, 1958; Goyal, 1985), BC are more numerous in the fifth segment than in any other segments of the epididymis. Their nuclei form a dense cell layer in this segment. Sinowatz (1981) reported that the BC exist in larger numbers in the bovine corpus epididymidis than in the caput epididymidis, where the ratio between them and the PC reaches 1: 3 in the region of the epididymal body. On the other hand, the fifth segment of the equine (Nicander, 1958) epididymal duct possesses large and distinctly less numerous BC than the previous segments. In the sixth segment the size of the BC is somewhat larger than in any of other epididymal segment. They, however, present similar morphological characteristics as in the previous segments. This has been found in previous studies (Erkmann, 1971; Sinowatz, 1981; Goyal, 1985). Nicander (1958) stated that the BC were more flattened and sparser in the ovine and equine epididymal tail region.

In addition to PC and BC, apical cells are another cell type which is distributed in certain epididymal regions in many mammalian species. The designation of this cell type with the term apical cell is confusing (Martínez-Garcia et al., 1995) since this term has been used to designate different cell types in the mammalian epididymal duct including clear cells, apical mitochondria-rich cells (AMRC) and a subgroup of PC with apically located nuclei (Sun and Flickinger, 1980; Abou-Haila and Fain-Maurel, 1984). Furthermore, the AMRC have also been called with other names such as holocrine cells (Martan and Risley, 1963), narrow cells (Flickinger et al., 1978), flask cells (Abou-Haila and Fain-Maurel, 1984), club-shaped cells (Allen and Slatter, 1957), wineglass-shaped cells (Levine and Marsh, 1971) and mitochondria-rich goblet cells (Dacheux and Dacheux, 1988). In spite of the numerous studies on this cell type, the confusion created in its designation has hindered a better comprehension of its morphological and functional characteristics (Martínez-Garcia et al., 1995) and thus the designation of them as “apical mitochondria-rich cell” appears plausible. They were not found

in the first epididymal segment of the bovine (Goyal, 1985) or in the caprine (Goyal and Williams, 1991) epididymis. Similar findings could be reported in the present work. Furthermore, small numbers of them were found in some cross sections of the second segment (Sinowatz, 1981). They are found apical and possess narrow bodies, pale cytoplasm and a dome-shaped luminal border with few short microvilli. Their nuclei were round or oval and occupied the apical third of the cell (Sinowatz, 1981; Goyal, 1985). They were also found in the corresponding segment of the goat (Goyal and Williams, 1991), in the initial segment of the rat (Hamilton, 1975), hamster (Flickinger et al., 1978) and mouse (Soranzo et al., 1982), in regions II-IV (head and body) of guinea pig (Hoffer and Greenberg, 1978) and in the initial segment, head and body of the monkey epididymis (Ramos and Dym, 1977a). AMRC exist frequently in the third segment of the bovine epididymal head (Sinowatz, 1981; Goyal, 1985). They are encountered only rarely in the body region and when found they present a similar morphology as in previous segments. AMRC were not found in the epididymal tail region of the bull (Sinowatz, 1981; Goyal, 1985) or of the cat (Sanchez et al., 1998). In most species, including mouse (Burkett et al., 1987), rat (Brown and Montensano, 1980), hamster (Flickinger et al., 1978), and human (Palacios et al., 1991), AMRC are more abundant in the caput than in the corpus and are rare in the cauda epididymidis. In monkey (Ramos and Dym, 1977a) and bull (Goyal, 1985) they are more numerous in the distal portion of the caput and along the whole corpus region and are absent in the cauda epididymidis. However, AMRC are very rare in all epididymal regions in guinea pig (Hoffer and Karnovsky, 1981).

Intraepithelial lymphocytes (IEL) are seen throughout the entire length of the epididymis. As recorded in previous investigations (Sinowatz, 1981; Goyal 1985), IEL are found mainly near the basal membrane and occasionally at higher levels. Similar findings were also reported in the goat epididymis (Goyal and Williams, 1991).

Intraepithelial macrophages (IEM) are also found throughout the entire length of the epididymal duct. Similar findings were recorded by Nicander (1958), Erkmann (1971), Sinowatz (1981) and (Goyal, 1985) in the bovine epididymis and by Nicander (1958) in the ram and by Goyal and Williams (1991) in the goat. Furthermore, Erkmann (1971) described these cells in the histological sections stained with ordinary stains to have dark irregular eccentrically located nuclei surrounded by an unstained space. These cells were found along the entire length of the bull epididymis and when stained with PAS they express coarse diastase-resistant PAS-positive granules. These cells occur near the basal membrane of the bovine epididymal epithelium throughout its entire length (Sinowatz, 1981; Goyal, 1985). In

accord with Nicander (1958) and Sinowatz (1981), I found numerous IEM containing PAS-positive granules in the third segment. Their number in the third segment was higher than in the previous two segments. Nicander (1958) found also numerous IEM in the corresponding segment of the ovine but not of the equine epididymis. In agreement with Sinowatz (1981), the numbers both of IEL and IEM decrease in the fourth and fifth segment of the epididymis. Both of IEL and IEM existe in variable numbers in the neighbouring tubules of the same section of the sixth segment (Sinowatz, 1981; Goyal, 1985). Furthermore, IEM are more numerous in the sixth segment than in any other segment. On the other hand, in stallion epididymal tail region (Nicander, 1958) the IEM were much less numerous than in the fifth segment.

Typical intraepithelial glands were early described by Nicander (1958) in the first segment of the head of the bovine epididymis. Later on, Tröger (1969), Erkmann (1971) and Sinowatz (1981) described these structures as deep epithelial crypts. In the present work similar structures were found in the first epididymal segment. Similarly, epithelial crypts lined by cells closely resembling the PC were found in epididymis of camel (Singh and Bharadway, 1980), cat (Arrighi et al., 1986) and stallion (Arrighi et al., 1993). Although no plausible interpretations of their functional significance are available, my immunohistochemical studies showed that their luminal surfaces express strong immunoreactivity for both S-100 and ACE. Both protein fractions possess a well-known role in the regulation of fluid and electrolyte transepithelial transport (Molin et al., 1985; Zhao et al., 1996; Leung et al., 1999; O'Mahony et al., 2000). However, further investigations are necessary to shed light on their definite physiological role.

The peritubular muscle coat (PMC) increases gradually from 26.01± 1.76 µm in the first segment to 55.60 ± 9.05 µm in the sixth segment. This was in accordance with previous studies (Erkmann, 1971; Sinowatz, 1981; Goyal, 1985; Lindinger-Niederhofer, 1991). The PMC reached its maximal thickness in the epididymal tail region in all the species studied (Nicander, 1958; Holstein, 1969; Wrobel and Fallenbacher, 1974; Tingari and Moniem, 1979; Chandler et al., 1981; Abdou et al., 1985; Goyal and Williams, 1991; Delhon and Lawzewitsch, 1994; Sanchez et al., 1998, Ruhl, 2001). This elucidates the role of PMC of the sixth segment in the ejaculation.

5.2 Lectin binding sites in bovine epididymis

FITC- or horseradish peroxidase-labelled lectins are extensively used in histochemical localization of reactive saccharide groups in tissue sections (Watanab et al., 1981; Gabius et

al., 1988). Among the ten lectins (HPA, VVA, UEA-I, LTA, PSA, DBA, LCA, WGA, PNA and GSA-I) used for localization of sugar residues in prenatal epididymis, only three lectins (WGA, PNA and GSA-I) possessed binding sites in the epithelium of ED. The binding sites are mainly restricted to their apical surface. WGA-binding sites are restricted to the apical surface of the epithelium of the ED from CRL 36 cm (140 pcd, 5th gestational month) and upwards. Connective tissue and peritubular cells are WGA-positive both in ED and epididymis. PNA-binding sites are restricted to the apical surface of the epithelium of ED from CRL 10 cm (75 pcd, 3rd gestational month) and upwards. GSA-I-binding sites display a reaction pattern similar to that of PNA, but the blood vessels reveal a positive reaction. In contrast to the condition in the ED, the foetal epididymal epithelium does not express binding sites for any lectin. Lectin histochemistry can demonstrate changes in various biological processes including embryonic differentiation and cell maturation (Lis and Sharon, 1986). Furthermore, the epithelial cells lining the ED of the bovine foetus undergo cytological differentiation at about the third month of gestation. This differentiation proceeds in a proximodistal direction (Wrobel, 2001). Lectin-binding sites both in the foetal ED and epididymal epithelium suggest that the epithelium of the ED differentiate earlier than that of the epididymal duct.

With the exception of two L-fucose-binding lectins (LTA and UEA-I) and an N-acetyl-D-galactosamine-binding lectin (VVA), all the used lectins (GSA-I, PNA, ECA, WGA, Con A, LCA, PSA, DBA, HPA, SBA) exhibit a characteristic pattern of binding sites in the adult bovine epididymis. On the other hand, Prem (1992) and Kuhrau (1993) found marked binding sites for UEA-I in the feline and equine epididymis respectively. Similarly, Lee and Damjanov (1984) recorded extensive binding sites for LTA in the mouse epididymis. This variation in reaction with L-fucose-binding lectins may be ascribed to species-specific differences (Arya and Vanha-Perttula, 1986). The N-acetyl-D-galactosamine-binding lectin VVA did not react with any portion of the epithelium neither of ED nor of epididymal duct. This was in accordance with the results of Lee and Damjanov (1984), who found no binding sites for VVA in the mouse epididymis.

WGA is an N-acetyl-D-glucosamine- and sialic acid-binding lectin. In my study WGA-binding sites were found in nonciliated cells of the ED. This is in accordance with Wakui and co-workers (1996), who reported WGA-binding sites in nonciliated cells of the dog ED. Lectins belonging to D-mannose/D-glucose-binding group also expressed binding sites in nonciliated cells of bovine ED. This group of lectins includes Con A, LCA and PSA. ED expressed an alternation between positive nonciliated and negative ciliated cells with LCA,

PSA and Con A. Apical surface and protrusions reacted stronger. My findings were in accordance with those obtained by Kuhrau (1993), who found Con A-binding sites in nonciliated but not ciliated cells of the equine ED. However, Prem (1992) found Con A-binding sites both in ciliated and nonciliated cells of feline ED. N-acetyl-D-galactosamine-binding lectins expressed different pattern of the distribution. HPA and SBA two members of this group exhibited binding sites on the apical surface of ED in a patchy pattern. A similar reaction was expressed by luminal contents. On the other hand, DBA another member of this group showed no binding sites in ED. Prem (1992), Kuhrau (1993) and Wakui and coworkers (1996) reported similar results regarding the localization of SBA-binding sites in the feline, equine and canine ED respectively. They also found binding sites for DBA in the ED of the species they investigated. Lectins (GSA-I and PNA) with specific affinity to D-galactose exhibited a different pattern of binding sites in ED. GSA-I revealed binding sites in the apical surface and apical half of the epithelium, whereas PNA did not show any binding sites in the epithelium of ED. My lectin studies demonstrate that the bovine ED display a distinct distribution of lectin binding sites that is different from other domestic mammals like cat (Prem, 1992), dog (Wakui et al., 1996) and horse (Kuhrau,1993).

The epithelium of bovine epididymis revealed great regional variation in the lectin binding sites expressed by the stereocilia. Stereocilia of PC in the head region exhibited markedly positive reaction with Con A, WGA, GSA-I, ECA, PSA, LCA, HPA and DBA. In the more distal segments, stereocilia reacted positively only with Con A, WGA, PSA and LCA. Arya and Vanha-Perttula (1985a) and Rauscher (1991) recorded similar results in the same species. The regional differences in the distribution of lectin-binding sites on the stereocilia of the epididymal epithelium lend a support to the findings reported by Sinowatz (1981). He found that the stereocilia of PC in caput region display a distinct activity of alkaline phosphatase (ALPase), whereas those of the more distal segments were ALPase-negative. Goyal and Hrudka (1980) reported that marked ALPase-activity reflect absorptive function of the cells. It is noteworthy that most of the rete testis fluid is reabsorbed in the region of the caput epididymidis (Carbo, 1965; Dacheux et al., 1989). Moreover, Sinowatz (1981) proposed that the characteristic enzyme content of the stereocilia in the different segments reflect their specific absorptive activity in each segment.

There is a marked regional variation regarding lectin-binding sites expressed by the Golgi apparatus of the PC throughout the epididymal duct. The Golgi apparatus of the PC of the proximal three (head) segments bind intensely with WGA, HPA, DBA and GSA-I. These results agree with those of Rauscher (1991), who found binding sites for WGA, DBA and

GSA-I in the bovine epididymis. This author found also binding of Con A in the Golgi apparatus in this region. In the present work, Con A-binding sites could not be localized in Golgi zone since the PC expressed a diffuse Con A-binding in the cytoplasm of the whole cell. Arya and Vanha-Perttula (1985a) also found binding sites for Con A but not for DBA in the Golgi zone (GZ) of the PC in the same species.

In accordance with the findings of Rauscher (1991) and Arya and Vanha-Perttula (1985a) PNA-binding sites could not be localized in the GZ of bovine PC. Different to the study of Arya and Vanha-Perttula (1985a), SBA-binding sites could not be localized in GZ of bovine PC. The lectin-binding sites in the GZ of the PC may reflect the indispensable role of this organelle in the great secretory and resorptive potential of these cells. Our ultrastructural study reinforces this assumption. This also could be proved by Sinowatz (1981) who found an extensive Golgi apparatus in the proximal three (head) epididymal segments. He mentioned that Golgi apparatus is not only concerned with production of secretory granules but also with synthesis of membrane glycoproteins and lysosomal enzymes. Golgi apparatus represents the subcellular site for glycosylation of glycoproteins and glycolipids (Ramakrishnan et al., 2001). The PC which possessed positively reacted GZ expressed also lectin staining of the apical cytoplasm and luminal border. This was in accordance with the findings of Arya and Vanha-Perttula (1985a) who ascribed these binding sites to the secretory process. Similarly, Rauscher (1991) interpreted the extensive distribution of lectin-binding sites in the GZ and apical areas of PC as an indication of the high secretory activity of the PC in the epididymal head region. It is noteworthy that many of epididymal secretory products involved in sperm maturation are glycoproteins (Faye et al., 1980). In the middle (body) region, I found binding sites for WGA, HPA, PSA and LCA. WGA-binding sites have been localized in the GZ of the PC in the body region of bovine epididymis in the studies performed by Arya and Vanha-Perttula (1985a) and Rauscher (1991). HPA-binding sites were recorded in the GZ of PC throughout the entire length of the epididymal duct of the boar (Calvo et al., 2000). In accordance with the results reported by Sinowatz (1981), my ultrastructural study of the PC in the body region of the bovine epididymis revealed smaller Golgi apparatus than that of the head region. This may point to a somewhat reduced secretory activity in this region of the epididymis (Sinowatz, 1981; Rauscher, 1991). Furthermore, Sinowatz (1981) mentioned that Golgi apparatus in this part of the duct may be involved in the synthesis of lysosomal enzymes. Different from the results of Arya and Vanha-Perttula (1985a) and Rauscher (1991) no definite binding sites for Con A and WGA in the GZ of the PC could be found in the cauda epididymidis.

In agreement with previous studies (Arya and Vanha-Perttula, 1985a, Rauscher, 1991), a continuous rim of lectin (Con A, WGA, HPA, PSA, and LCA)-stained materials was found at the basal portion of the PC in regions II through VI of the bovine epididymis. My electron microscopic studies on these segments of bovine epididymis showed residual bodies and heteromorphic granules in the basal extensions of the PC around the BC. These heteromorphic granules may be lysosomal in nature (Sinowatz, 1981) and could be used in the digestion of internalized material.

The BC expressed lectin-binding sites for Con A, WGA, HPA, PSA and LCA throughout the entire length of the epididymal duct. The intensity of reaction however, increased from the caput to the cauda epididymidis. In other studies, similar binding sites for WGA and Con A were recorded in the BC of bovine (Arya and Vanha-Perttula, 1985a; Rauscher, 1991), feline (Prem, 1992) and equine (Kuhrau, 1993) epididymis. Arya and Vanha-Perttula (1985a) ascribed this distribution of lectin binding sites in the basal portions of the PC and in BC to their co-operation in absorption and elimination of certain materials from the epididymal lumen. Rauscher (1991) showed a similar trend in this regard. They assume that the PC are involved in transporting absorptive materials to IEM, which possess numerous acid phosphatase-positive globules (Sinowatz, 1981). They take over and degrade the resorbed material and migrate back into the interstitium (Sinowatz, 1981). He assumed that IEM can clear the epididymal epithelium of indigestible waste material. It is noteworthy that I found IEM exhibited binding sites for PNA, WGA, LCA and PSA. My findings are in accordance with those of Lindinger- Niederhofer (1991) who reported WGA-binding sites on the IEM in the bovine epididymis. Furthermore, Arya and Vanha-Perttula (1985a) found macrophage-like cells expressing SBA and UEA-I-binding sites in the interstitial tissue of bovine epididymis. In agreement with the findings of Sinowatz (1981) and Goyal (1985), I found numerous heteromorphic granules in the basal extensions of the PC around the BC. Sinowatz (1981) mentioned that these granules are derived from Golgi apparatus and possess high activity of many hydrolytic enzymes which may support their active role in phagocytic and digestive functions.

Although AMRC were stained by WGA, UEA-I, Con A, SBA and PNA in many mammalian species (Arya and Vanha-Perttula, 1984; Burkett et al., 1987; Lee and Damjanov, 1984; Prem, 1992; Calvo et al., 1995, 2000), no distinct reactivity with any of the lectins used was found. Arya and Vanha-Perttula (1984) reported that AMRC were Con A-, PNA- and SBA-positive in the Bouin-fixed rat epididymis. I also studied Bouin-fixed material, however no binding

sites could be found in my work. Thus, I assume that this might be due to species differences (Arya and Vanha-Perttula, 1986).

The basal membrane (BM) of the epididymal epithelium revealed binding sites only for WGA. Rauscher (1991) found no binding sites for any of these lectins used in the BM of the bovine epididymis, whereas Arya and Vanha-Perttula (1985a) found binding sites for the same lectins in the same species. In other species including cat (Prem, 1992) and horse (Kuhrau, 1993) WGA-binding sites could be demonstrated in the BM of the epididymal duct. In agreement with previous studies (Arya and Vanha-Perttula, 1985a; Rauscher, 1991), the connective tissues surrounding the epididymal duct presented a weak reaction with Con A, LCA, PSA, SBA, WGA, DBA and ECA.

In accordance with the findings of Rauscher (1991), the vascular endothelium revealed binding sites for Con A, PSA, LCA, HPA, WGA, ECA and GSA-I. The latter expressed the highest intensity. GSA-I can be regarded as a marker for bovine endothelial cells. Kuhrau (1993) recorded a similar pattern of vascular distribution of binding sites for Con A, WGA and GSA-I in the endothelium of equine epididymis. Furthermore, Prem (1992) found Con A- and WGA- binding sites in the vascular endothelium of the feline epididymis.

Binding sites for Con A, WGA, LCA, PSA, ECA, HPA and PNA could be localized on the epididymal spermatozoa. No binding sites for SBA, GSA-I, DBA, VVA, LTA or UEA-I could be found. Similar results were recorded by Rauscher (1991) who found binding sites for Con A, WGA and PNA but not for GSA-I, DBA, SBA or UEA-I in the same species. The pattern of reaction expressed no marked variation among the different epididymal segments. This was in agreement with the results of Rauscher (1991). However, Arya and Vanha-Perttula (1985a) reported that the bovine spermatozoa expressed some changes in their affinity to lectins along the epididymal duct. They mentioned that sperms in epididymal head region exhibited a strong binding to Con A and WGA. The intensity of this reaction fades toward the cauda epididymidis, whereas the intensity of PNA-binding increased. Furthermore, my lectin studies showed that the bovine spermatozoa displayed a distinctive distribution of lectin binding sits that is different from other mammalian species including cat (Prem, 1992), boar (Zücher, 1992; Calvo et al., 2000) and horse (Kuhrau, 1993).

Although some lectins possess the same monosaccharide-specificity, they exhibit very different distribution pattern of their binding sites. The N-acetyl-galactosamine-binding lectins (DBA, HPA SBA and VVA) exhibited remarkable differences not only in the number of binding sites, but also in their pattern of distribution. Similar findings were reported by Rauscher (1991) in the bovine epididymis. This might be ascribed to non-covalent binding to

the tertiary structure of the receptors as well as to the strict binding to the entire spatial structure (Ozawa and Muramatsu, 1985). Thus lectins with identical monosaccharide-specificity can identify differences in structural complexity of receptors (Kunz et al., 1984). Moreover, binding of a lectin to its specific monosaccharide may be affected by several factors such as influence of the neighbouring sugars. Thus lectins with the same nominal sugar specificities are not necessarily bound to the same glycoconjugates (Malmi and Söderström, 1988).

The method of fixation employed clearly influence the lectin binding to tissue sections (Malmi and Söderström, 1988). However, I found no significant difference between the Bouin-fixed and the frozen sections, post-fixed in acetone. Moreover, I found that Bouin-fixed sections surpass the frozen sections in the preservation of their morphology. Although Lee and Damjanov (1984) have used a different fixative, they also found no difference between lectin-binding sites in paraffin-embedded and frozen sections of the mouse epididymis. Decreased staining of tissue structures with many lectins after fixation in formaldehyde might be ascribed to its binding to hydroxyl groups at positions responsible for the binding of a particular lectin (Malmi and Söderström, 1988). Apart from poor preservation of the tissue morphology, the use of frozen sections with acetone fixation is the ideal tissue preparation method for lectin histochemistry (Malmi and Söderström, 1988).

5.3 Immunohistochemistry

5.3.1 S-100 protein

S-100 protein appears in a subcellular fraction first isolated from bovine brain. It was called S-100 due to its solubility in a 100 % saturated solution of ammonium sulphate at neutral pH (Moore, 1965). Immunoreaction against S-100 protein begins to appear in the bovine ED and epididymal duct at the foetal age of about 75 pcd (CRL, 10 cm) and progressively increases with advance of foetal age. Immunoreactivity for S-100 proteins was also detected on the apical surface of the PC and weakly in the BC of the human epididymis (Haimoto et al., 1987). This agrees with my results in the bovine. S-100 has been associated with several activities including regulation of the diffusion of monovalent cations across membranes and modulation of the physical state of membranes (Zimmer et al., 1995). Furthermore, S-100 proteins may be involved in the processes regulating pH, electrolyte and water content of the kidney tubular cells (Molin et al., 1985). I assume that S-100 may promote a similar task in

the excurrent duct system of the male genital tract. In the present work also, the vascular endothelium expressed a weak to moderate immunoreaction for S-100 proteins. Similar findings were reported in the endothelial cells of arteries, veins, and lymphatic sinusoids of bison (Czykier et al., 1999) and bovine (Amselgruber et al., 1992) epididymis. Immunoreactivity for S-100 was also observed in the endothelia of capillaries of rat and mouse epididymis (Czykier et al., 2000). Furthermore, endothelial cells of capillaries, veins and lymphatic vessels were regularly S-100 immunoreactive in ruminants (Amselgruber et al., 1994). It was therefore assumed that it may participate in the processes of transcytosis and cell contractility (Czykier et al., 2000).

5.3.2 Fibroblast Growth Factors (FGFs)

FGFs are multifunctional peptides with a potent mitogenic activity for most of the mesodermally derived cells and many cells of ectodermal origin (Mellin et al., 1992), which are involved in embryogenesis, tissue differentiation, angiogenesis, wound repair and carcinogenesis (Dorkin, 1999). The acidic FGF (aFGF = FGF-1) and the basic FGF (bFGF = FGF-2) are the first two FGFs that were identified and extensively characterized (Mellin et al., 1992). These locally produced factors act in a paracrine or autocrine manner to regulate cellular activities (Rifkin and Mascotelli, 1989). FGFs interact with high affinity cell surface receptors identified on many cell types (Hughes and Hall, 1993). FGFs interact with membrane associated tyrosine kinase high-affinity receptors (FGFRs) and with heparan sulfate glycosaminoglycan (HSGAG) low-affinity receptors (Hughes and Hall, 1993). FGFs mediate their biological effects through their interaction with the high affinity specific surface receptors (Basilico and Moscatelli, 1992, Hughes and Hall, 1993).

Changes may take place in FGF receptors (FGFRs) expression levels during proliferation, differentiation or malignant transformation (Bikfalvi et al., 1997b). The biological response of cells to FGFs is mediated by the high affinity receptors (Fernig et al., 1992); however, heparan sulphate low-affinity receptors may be necessary for the activation of FGFs (Fernig et al., 1992). FGFs / FGFRs interactions play major roles in various developmental processes (Kirby et al., 2003) involving mesoderm induction and the maintenance of mesoderm during gastrulation (Bikfalvi et al., 1997b), integration of growth, budding and patterning during early post-implantation, as well as the development of various tissues (Coulier, 1997).

FGFs regulate a wide variety of biological activities including control of expression and deposition of extracellular matrix components, angiogenesis, development, tissue or wound repair, and pathogenesis of several diseases especially malignant transformation (Burgess and

Maciag, 1989; Baird and Bohlen, 1990; Kusafuka et al., 1998; Dorkin, 1999). They have a chemotactic effect on fibroblasts and are able to promote the proliferation of them and of other mesoderm-derived cells (Burgess and Maciag, 1989; La Rosa and Chiaravalli, 1997). Both of FGF-1 and FGF-2 are immunolocalized in the peritubular smooth muscle cells of the human epididymis (Hughes and Hall, 1993). Smooth muscle cells express FGF-1, which may play a role in the induction of neovascularization (Bikfalvi et al., 1997b). In the present work, immunoreactivity for FGF-1 and FGF-2 was found as early as 75 pcd (CRL, 10 cm) both in the cells of the epithelium lining ED and the epididymal duct. This reactivity increased progressively with advance of the foetal age. The immunoreaction for FGF-1 was confined to the cytoplasm, whereas that for FGF-2 included both of cytoplasm and nuclei of the epithelial cells. At the CRL 13 cm (80 pcd), a positive FGF-2-immunostaining began to appear in the peritubular extracellular matrix, vascular endothelium and many cells of the connective tissue. It is evident that FGF-2 is required for growth and development of the epididymis (Alarid et al., 1991). The *in vivo* neutralization of FGF-2 suppressed the growth of the male genital ridge and inhibited the differentiation of the epididymis (Alarid et al., 1991). This inhibition is unlikely due to impairment of angiogenesis since all other derivatives of male genital ridges grew normally (Alarid et al., 1991). The extracellular matrix and the basement membrane surrounding the foetal mesonephric duct expressed FGF-2-immunoreactivity (Gonzalez et al., 1990). Thus FGF-2 may function as a mediator of the mesenchymal-epithelial interactions, particularly in the developing epididymis. FGF-2 induces differentiation both of haematopoietic and endothelial cells (Bikfalvi et al., 1997b). Thus bFGF may be important for embryonic vascular development through enhancing the endothelial cells proliferation (Bikfalvi et al., 1997b). Immunoreaction for FGF-1 in the epithelium lining ED exhibited an alternation between strongly positive ciliated cells and partially reactive nonciliated cells. Furthermore, FGF-1-immunoreaction was evident throughout the different regions of the epididymis. The immunoreactivity for FGF-2 in the bovine ED was represented by a weakly positive cytoplasm as well as strongly positive nuclei of the ciliated cells. The latter can presumably enhance proliferation, growth and differentiation of neighbouring nonciliated cells via a paracrine mechanism. Apart from some minor differences, the epithelium lining the different epididymal segments showed a similar pattern of immunoreactivity. The latter was represented by a strong signal in the nuclei of the basal cells along the whole length of the duct. On the other hand PC were absolutely negative for FGF-2. However, specific FGF-2-receptors were found on the PC of the epididymis (Kirby et al., 2003). Thus, I propose that FGF-2-positive BC play a role in the proliferation, growth and differentiation of the PC via a

paracrine mechanism at least in the bovine epididymis. The nuclei of the peritubular SMC and the vascular endothelial cells and MC in the interstitium displayed a moderate to strong immunoreaction. MC can synthesize and secrete VPF/VEGF. Furthermore, FGF-2 and VEGF were immunolocalized in the mature tissue MC (Grützkau et al., 1998). The epididymis is not an active site for angiogenesis in adults (Lissbrant et al., 2003) and FGF-2 controls VEGF levels (Bikfalvi et al., 1997a). VEGF exerts its biological role in the epididymis via regulation of vascular permeability (Lissbrant et al., 2003). Moreover, FGF-2 could improve testicular blood flow following ligation of testicular vessels (Guler et al., 2004). Thus, I assume that FGF-2 play a role in regulation of epididymal blood flow. Furthermore, it modulates the function of Leydig cells. The latter are the main producer of androgens (Wrobel, 1998), which are essential for epididymal functions (Arya and Vanha-Perttula, 1985b).

5.3.3 Angiotensin Converting Enzyme (ACE)

ACE is a membrane-bound glycoprotein, which is detectable in all tissues and body fluids of mammals (Suffer, 1976). It plays a key role in the renin-angiotensin-aldosterone system (Peach, 1977), which is engaged in blood pressure regulation and volume homeostasis. ACE catalyses the formation of the physiologically active octapeptide angiotensin II from the inert decapeptide angiotensin I in many organs including the male reproductive tracts (Cushman and Cheung, 1971; Wong and Uchendu, 1990, 1991). There is an intracellular pathway for angiotensin II synthesis and thus an endogenous or local renin-angiotensin system in many tissues and organs including the male (Pandy et al., 1984) and female (Lightman et al., 1987) genital organs.

Two isoforms of ACE are produced in male mammals, a somatic (sACE) and testicular or germinal (gACE) isoforms. The germinal isoform is exclusively expressed in the male haploid germ cells (Sibony et al., 1994). Bradykinin is an ACE-substrate, which plays a role in sperm motility (Schill and Haberland, 1974). ACE has been correlated to sperm maturation (Cushman and Cheung, 1971), which may be attributed, to inactivation of kinins and thus suppresses the intraepididymal sperm motility or modifying the constituents of the sperm plasma membrane (Vanha-Perttula et al., 1985). Early occurrence of ACE in the epithelial cells of the mesonephros and Wolffian duct has already been recorded (Schütz et al., 1996). Furthermore, the protein of sACE is found at the adluminal membrane of epithelial cells lining proximal mesonephric tubules and Wolffian duct and thus corresponds very early to the final expression sites of adults (Pauls et al., 2003). Similar findings were recorded in my work, since the immunoreaction for ACE appears as early as 75 pcd (CRL, 10 cm) and is

restricted to the apical cytoplasm and surfaces of the ED and the proximal portion of the growing epididymal duct and even in the degenerating mesonephric structures corresponding to the epididymal body region. Since it stimulates angiogenesis in vivo (Fernandez et al, 1985) and acts as a growth factor (Naftilan et al., 1989) in cell culture systems, ACE may play a role in the development and differentiation of the extratesticular male genital tract. It was concluded that the presence of components of renin-angiotensin system and specific receptors for angiotensin II both in the male and the female reproductive tracts, supports the hypothesis that renin-angiotensin system can control the reproductive functions (Köhn et al., 1998). Several studies showed release of ACE from human spermatozoa during capacitation (Köhn et al., 1995) and reported its significance for fertilization process (Köhn et al., 1998). The existence of a renin-angiotensin system in the epididymis of rat may interpret the role of ACE released by epididymal spermatozoa in regulation of epididymal function and sperm maturation (Leung et al., 1999). ACE converts the locally produced angiotensin I (by epididymal epithelial cells) into angiotensin II. The latter plays a paracrine role through regulating electrolyte and fluid transport in the epididymis (Zhao et al., 1996; Leung et al., 1999; O'Mahony et al., 2000).

The effects of angiotensin are mediated via angiotensin receptors (Vinson et al., 1997). The effect of angiotensin II may be mediated through their paracrine and/or autocrine regulatory interactions via their receptors on both apical and basal surfaces of epididymal epithelium (Leung et al., 1997). In the present work the immunoreaction for ACE was localized in the vascular endothelium along the length of ED and epididymal duct. Furthermore, a strong immunoreactivity was found on the luminal surface as well as in the supranuclear area of many nonciliated cells lining ED. Both of apical surfaces and kinocilia of ciliated cells showed a similar reaction. Similar findings were reported by Berg and co-workers (1986) in rabbit and by Vivet and co-workers (1987) in human epididymis. They found primary cellular localization of ACE in vascular endothelial cells adjacent to the lumen. In addition to vascular endothelium in human male genital tract, immunoreactivity for ACE was observed on the luminal surface of the epithelium of the ED, epididymis, and ductus deferens especially in stereocilia (Vivet et al., 1987). Furthermore, the immunoreactivity for ACE in the epididymis is low in the initial segment, highest in the head and moderate in the tail of rat epididymis (Strittmatter et al., 1985). Since the ACE-immunoreactivity was restricted to stereocilia and the apical cytoplasm of the first two segments of the bovine caput epididymidis and in supra- and infranuclear cytoplasm of scattered PC in the sixth segment, my findings are in accord with those of Strittmatter and Synder (1984), who found particulate immunoreactivity for

ACE on the epithelial cells of the rat caput and cauda epididymidis. On the other hand, intense reaction has been located on the luminal surface both of human corpus and cauda but not of caput epididymidis. It was strictly confined to the stereocilia and immunoreactivity was not seen in the cytoplasm of PC or BC (Vivet et al., 1987).

5.3.4 Vascular Endothelial Growth Factor (VEGF)

VEGF is a heparin-binding growth factor specific for vascular endothelial cells (Leung et al., 1989). It is a potent angiogenic inducer that has been implicated in physiological and physiopathological conditions associated angiogenesis (Armesilla et al., 1999). Thus it plays important roles during tumour angiogenesis (Hetian et al., 2002). Since it has the ability to induce vascular leakage in the guinea pig skin (Connolly et al., 1989), VEGF has the synonym vascular permeability factor (VPF) and accordingly it increases the microvascular permeability as a crucial step in angiogenesis associated with tumours and wounds (Ergün et al., 1997). The permeability potency of VEGF is 50,000 times greater than that of histamine (Connolly et al., 1989). This increased vascular permeability may be due to the induction of fenestrations in the endothelium of small venules and capillaries, even in regions where endothelial cells are normally not fenestrated (Roberts and Palade 1995). It exerts its activities through binding to receptors kinases VEGFR-1 and VEGFR-2. In disagreement with our results, VEGF was expressed in several cell types in the human male genital system including Leydig cells and Sertoli cells of the testis as well as certain epithelial and peritubular cells of the epididymis (Ergün et al., 1997; 1998). In the present work immunoreaction for VEGF was found only in mast cells (MC) in the epididymal interstitum, particularly next to blood vessels. MC release a variety of angiogenic factors (Crivellato et al., 2004), and therefore, the biological role of VEGF in the testis and epididymis may be associated with regulation of vascular permeability (Lissbrant et al., 2003). Furthermore, VEGF and consequently MC have regulatory effects on certain blood cells as for example, promotion of monocyte chemotaxis (Clauss et al., 1990). In addition to the chemotactic effect of MC on blood monocytes, the increased vascular permeability may result from the ability of VEGF in inducing fenestrations in the endothelium of small venules and capillaries (Roberts and Palade 1995). Both events may help migration of mononuclear cells from blood into interstitium and consequently into the epididymal epithelium. The present work reported existence of numerous $CD68^+$ cells (macrophages) both in the interstitium and within the epididymal epithelium. These $CD68^+$ macrophages along with T-lymphocytes participate in the induction of immune tolerance in the testis and its excurrent ducts (Marchlewicz, 2001).

5.3.5 Galactosyltransferase (GT)

Galactosyltransferase (GT) is a member of a functional family of enzymes that work co-ordinately in the biosynthesis of carbohydrate moieties of glycoproteins and glycolipids (Russo et al., 1990; Shaper et al., 1990; Ramakrishnan et al., 2001). GT catalyzes the transfer of galactose from UDP-galactose to the acceptor N-acetyl glucosamine (Shaper et al., 1990). Some GT are membrane-bound whereas others function as soluble enzymes (Hennet, 2002). The immunoreactivity for GT began to appear as early as at the foetal age of 75 pcd (CRL, 10 cm). The epithelial lining of the ED expressed supranuclearly a moderate positive cytoplasm. Furthermore, the epithelium of the epididymal duct exhibited also a positive supranuclear cytoplasm along the length of the duct. Moreover, stereocilia (epididymal duct) and cilia (ED) showed a positive immunoreaction for GT.

The immunoreaction for GT appeared to be mainly localized in the Golgi zone (GZ) of the PC of the adult epididymal epithelium and showed a marked variation in the different epididymal segments. This variation included both the intensity and the pattern of distribution. The PC of the epithelium lining the second and the third segment of the epididymal duct exhibited a strongly reactive supranuclear GZ. In the other segments GZ expressed a moderate to distinct immunoreaction. In addition to the GZ, a strongly reactive apical cytoplasm was found in the PC only of the second segment. Stereocilia demonstrated a moderate immunoreaction, but only in the PC of the first two segments, whereas those of the other segments were negatively. My results indicate that the segments of the caput epididymidis are the main site of synthesis and secretion of GT. This supports the findings of previous studies (Hoffer et al., 1973; Nicander and Malmqvist, 1977; Ramos and Dym, 1977a; Kohane, et al., 1980; López et al., 1989; Veeramachaneni et al., 1990; Liu et al., 1991; Arrighi et al., 1993; Cornwall and Hann, 1995), which indicated that the PC of the caput epididymidis are the main site of glycoprotein synthesis in different mammalian species.

5.3.6 α-Smooth Muscle Actin (α-SMA)

Contractile proteins, for example actin isoforms have been shown to be reliable differentiation markers (Osborn and Weber, 1983). α-SMA is mainly found in cells having contractile functions (Skalli et al., 1986). It is an isoform typical of smooth muscle cells (SMC) and is present in high amounts in vascular SMC (Skalli et al., 1989). A monoclonal antibody (Skalli et al., 1986) has been used for recognizing exclusively α-SMA and it appears to be a powerful probe in the study of SMC differentiation in normal and pathological conditions (Skalli et al.,

1986; 1989). In the present work, immunoreaction for α-SMA appeared as early as at the foetal age of 75 pcd (CRL, 10 cm), but only in a few periductular cells of some ED, which expressed a moderate reaction. On the other hand the peritubular cells surrounding the epididymal duct reacted negatively. The vascular SMC were almost strongly reactive especially in the larger vessels. At the foetal age of 95 pcd (CRL, 18cm), the periductular cells expressed a moderate immunoreaction for α-SMA in a concentric pattern surrounding the ED. The differentiating SMC surrounding epididymal duct began to exhibit a positive reaction. The intensity of the reaction was moderate to strong in the innermost layers, whereas the outer layer reacted in a weaker manner. In the following stages both the intensity and the distribution sites of the α-SMA-immunoreaction progressively increased with the advance of the age.

Cytodifferentiation of the peritubular cells is associated with a progressive increase of α-SMA-immunoreactivity (Francavilla et al., 1987). Immunostaining for α-SMA is spatially and temporally related to the appearance of contractile filaments (Francavilla et al., 1987), which appear first in cells just beneath the epithelium and progressively spread through more distant cells. My findings are in agreement with those of Francavilla and co-workers (1987) in this regard. A strong immunoreaction for α-SMA is acquired when cells appear ultrastructurally differentiated into mature contractile cells (Francavilla et al., 1987). Contrary to the condition in the bovine epididymal development, an immunoreaction for α-SMA begins to appear in newborn rat soon after birth. However, the pattern of distribution of immunoreactivity for α-SMA was similar in both species. In accordance with previous work (Francavilla et al., 1987), my findings showed that the differentiation of contractile cells in the epididymal wall is accompanied by progressive organization of the pericellular matrix into a continuous basement membrane, as discussed below (section 5.3.7; laminin). In the present work the cytoplasm both of periductal and vascular SMC in the adult bull showed a strong positive α-SMA-immunoreaction. Thus our findings agree with those reported by Francavilla and co-workers (1983) who found that the PMC of the adult rat epididymis express a strong α-SMA-immunoreaction. Since the passage of sperm through the epididymal duct is affected by the active contractions of the epididymis (Hamilton, 1975), the PMC play an important role in the epididymal function.

5.3.7 Laminin

Laminin is a member of a family of large extracellular matrix glycoproteins, which are abundantly present in basement membranes (Ekblom, 1996; Jucker et al., 1996; Hagg et al.,

1997). Laminin associates with other basement membrane components, such as nidogen/ entactin, type IV collagen and heparan sulfate proteoglycan. The basement membrane glycoprotein laminin-1 has been proven to be a particularly potent source of cell adhesion activity by its intimate association with the basal cell surface where it is associated with type IV collagen, perlecan and growth factors (Chen et al., 1997). Laminin has several biological activities (Engvall et al., 1990). It promotes cell attachment, spreading and motility (Haaparatna et al., 1991). The cellular interactions with laminin are important events that occur in a variety of physiological processes such as development, differentiation, cell migration and wound healing as well as in many pathological conditions including tumour invasion and metastasis (Romanov et al., 1994).

The reactivity for laminin in the bovine epididymis appeared in the 75 days old foetus (CRL, 10 cm). The overall reaction was moderate to strong in different tissue elements. The basal lamina both of ED and epididymal epithelium was strongly positive. Furthermore, the mesenchymal peritubular and interstitial cells as well as the vascular endothelial basal lamina exhibited a distinct positive immunoreaction for laminin. The intensity of immunoreactivity increased proportionally with the advance of foetal age. My findings are in accord with those of Paranko and co-workers (1985) who reported that the periductal accumulation of the basement membrane material reflects an incipient epididymal differentiation. Immunostaining for laminin and type IV collagen was seen around the Wolffian duct and mesonephric tubules before the beginning of gonad morphogenesis (Gelly et al., 1989). Moreover, my findings are in accordance with those of Paranko and co-workers (1985), who reported that the basement membrane surrounding the differentiating mesonephric duct was continuous as indicated from the positive immunoreaction for laminin and collagen IV.

In adult bovine epididymis, the basal lamina of the lining epithelium expressed a moderate to strong immunoreactivity along the length of the ED and the epididymal duct. The basal lamina of the SMC forming the PMC expressed a moderate immunoreaction. In accord with my findings, Gelly and co-workers (1989) reported that the differentiation of peritubular cells is accompanied by a progressive organization of the extracellular matrix into a continuous basement membrane during the postnatal maturation of rat epididymis. In agreement with previous work (Hagg et al., 1997), the vascular endothelium exhibited a moderate to strong laminin-immunoreactivity in all parts of the epididymis.

5.3.8 Connexin 43

Connexins (Cx) are members of a large family of integral membrane proteins with a common membrane topology (Thomas et al., 2002). They are the subunit proteins which oligomerize to form a hexameric hemichannel termed connexon (Willecke and Haubrich, 1996).

Two (connexons) hemichannels in apposed plasma membranes of neighbouring cells dock to each other and form an intercellular channel through which ions and metabolites can diffuse directly from cell to another (Roscoe et al., 2001). These intercellular channels are termed gap junctions (Bruzzone et al., 1996; Batias et al., 2000). The formation of gap junctions (GJ) in epithelial tissues involves both connexins and cell adhesion molecules (Cyr et al., 1996). The functions of GJ are diverse (Goodenough et al., 1996). They facilitate the co-ordination of individual cells in an organ (Evans and Martin 2002) and provide a mechanism for regulating the biological function of a whole tissue or an organ (Thomas et al., 2002). In nonexcitable cells, GJ may be involved in a wide variety of cellular activities including the regulation of development and differentiation (Bennett et al., 1991; Goodenough et al., 1996). Furthermore, the intercellular gap junctional communication plays a role in growth control and embryonic differentiation (Löwenstein and Azarania, 1988). In the present work no binding sites for connexin 43 could be found either in the epithelial lining or between the peritubular SMC both in ED and in epididymal duct. It is noteworthy that the concurrent testicular tissues in the same examined sections displayed a distinct immunoreactivity for connexin 43. Thus a technical error is absolutely excluded. My findings are thus in disagreement with those of Cyr and co-workers (1996) who stated that connexin 43 was immunolocalized at the apical margins between adjacent epithelial cells of the ED and at the base of the epithelium between BC and PC as well as between the peritubular SMC of the epididymis of rat (Cyr et al., 1996).

5.3.9 $CD4^+$ and $CD8^+$ T-lymphocyte

The bovine homologue of CD4 was first described by Baldwin and co-workers (1986) who mentioned that the CD4 antigen is expressed on the class II MHC-restricted helper T-lymphocytes. Furthermore, phenotype, tissue distribution and function of T cells expressing the CD4 antigen are similar to those reported in other species (Baldwin et al., 1986; Bensaid and Hadam, 1991). However, unlike findings in rat and man, expression of BoCD4 has not yet been detected on macrophages (Bensaid and Hadam, 1991). The CD8 antigen is expressed on the class I MHC-restricted cytotoxic T-lymphocytes (MacHugh and Sopp, 1991). It is a doublet and can exist as both a homodimeric and a heterodimeric form. The use of different

combinations of fixation, antigen retrieval (AR) and signal amplification has achieved successful immunostaining using some antibodies which in previous studies had yielded negative results in fixed tissue sections (Gutierrez et al., 1999). The use of formalin-dichromate fixation proved effective in preserving epitops such as CD4 and CD8. Similarly, the use of the AR method of microwave for unmasking the epitops yielded successful recovery of CD4, but not CD8. Also the use of the AR method of protease yielded positive results with both CD4 and CD8 (Gutierrez et al., 1999). In the present study, the CD4 could be localized in Bouin-fixed paraffin-embedded epididymal tissues without AR, whereas the CD8 could not be localized with the same technique. In the present work $CD4^+$ T-cells showed a positive reaction both in ED and in the different segments of the epididymal duct. They were found at different levels of the epithelium, but most frequently near the basal membrane. Similar $CD4^+$ cells were encountered in the interstitium and rarely in the lumina. On the other hand, no immunostaining for $CD8^+$ T-cells could be seen either in the ED or in any of the epididymal segments. This does not exclude the existence of $CD8^+$ T-cells in these tissues. Since our materials are Bouin-fixed, paraffin-embedded and have not been subjected to any of AR pretreatment, the negative reactivity might be ascribed either to masked or damaged epitops. This agrees with the findings of (Pileri et al., 1997) who reported that the formalin-fixed paraffin-embedded tissue is generally regarded as unsuitable for the well characterized cellular differentiation epitops and recommended the use of the frozen material (Pileri et al., 1997). Moreover, the CD4 and CD8 epitops are proved to be the most difficult to immunostain particularly, when they are found in formalin-fixed tissues (Rathkolb et al., 1997). In the rat epididymis, both of $CD4^+$ and $CD8^+$ T-cells are clearly more numerous in the basal region than in more apical locations and the $CD4^+$ are relatively more numerous than the $CD8^+$ T-cells (Flickinger et al., 1997). However, lymphocytes are never found in the lumen in any region of the epididymis (Flickinger et al., 1997). The existence of IEL was recorded both ultrastructurally (Wang and Holstein, 1983; Marchlewicz, 2001) and immunohistochemically (Marchlewicz, 2001) in human epididymis. In contrast to the testis, normal epididymal epithelium harbours many IEL (Nashan et al., 1990). IEL in the epididymis are under stringent control to avoid autoimmune destruction of spermatozoa (Pöllänen and Cooper, 1994). It is evident that any T-cell can function as a suppressor under appropriate conditions (Melchers, 1987). $CD4^+$ and $CD8^+$ T-cells are important regulatory elements governing the suppression of autoimmunity in mice (Itoh et al., 1992). Though this function is no longer strictly associated with $CD8^+$ T-cells (Mahi-Brown et al., 1988), it has been reported that the majority of lymphocytes found in the normal human testis and epididymis are suppressor/

cytotoxic $CD8^+$ T-cells (El-Demiry et al., 1987). On the other hand, also the murine $CD4^+$ T-cell, previously considered as helper/inducer cells, could be cytotoxic to targets bearing class II MHC molecules (Itoh et al., 1992). Furthermore, $CD4^+$ T-cells have been described by Flickinger and co-workers (1997) as regulatory lymphocytes, which play a role in suppressing autoimmune responses to sperm antigens that normally leak from the rat epididymal duct. On the other hand, Nashan and co-workers (1989) reported a very small number of suppressor/ cytotoxic T-cells with apparent lack of contact with the lumen in mice epididymis and mentioned that this contradicts the hypothesis of T-cell mediated immune suppression as a mechanism of tolerance.

5.3.10 $CD68^+$ macrophage

CD68 (EMB 11) is a macrophage marker which gives a strong positive reaction following AR using protease digestion (Gutierrez et al., 1999). $CD68^+$ cells were mostly seen in the basal region and rarely at higher levels of the epididymal epithelium along the length of the duct. Intraluminal $CD68^+$ cells were only seen in some tubules of segment III in an individual animal. The number of the positive cells was higher in segments II, III and VI than in other segments. However, the number of interstitial $CD68^+$ cells is very low in comparison to that of the intraepithelial macrophages (IEM) along the length of the duct. In contrast to the condition in epididymal segments, no $CD68^+$ cells could be localized in ED neither in the epithelium nor in the interstitium. In contrast to the findings of my work, $CD68^+$ cells were found mainly in the interstitium of the human epididymis (Marchlewicz, 2001). The absence of immunostained cells in the epithelium and interstitium of ED might indicate different antigenic phenotypes of macrophages in this region (Marchlewicz, 2001). In accord with my findings in the bovine epididymis, IEM are more numerous in the cauda epididymidis than in any other region of the human epididymis (Wang and Holstein, 1983). Flickinger and co-workers (1997) reported that IEM are nearly absent in the rat epididymis, however interstitial macrophages are more numerous, particularly in cauda epididymidis. IEM had no contact with the lumen (Nashan et al., 1989; Flickinger et al., 1997). However, I found $CD68^+$ cells in the epididymal lumen. Similar cells have been found in the human epididymal lumen (Wang and Holstein, 1983). Macrophages are involved in the digestion of sperm, bacteria or neoplastic cells and in the presentation of antigens to lymphocytes (Nashan et al., 1989). The abundance of macrophages in the immunologically privileged testis could possibly be involved in the creation of immune privilege status (Pöllänen et al., 1990). They may prevent

the exposure of autoantigens from physiologically degenerating germ cells to $CD4^+$ lymphocytes (Pöllänen et al., 1990).

5.4 Ultrastructural characteristics of bovine epididymis

5.4.1 Efferent ductules (ED)

The epithelium lining the bovine ED is simple columnar. It consists of ciliated and nonciliated cells, which are two different categories of columnar cells (Wrobel, 1972). In agreement with Goyal and Hrudka (1980; 1981) and based upon the cytological characteristics, nonciliated cells could be categorized into three subtypes which corresponded to the types described by them. Type I cells (contain neither granules nor vacuoles), type II cells (abundant in granules) and type III cells (abundance of vacuoles). On the other hand, the nonciliated cells exist in two functional stages (Wrobel, 1972). He mentioned that the apices of the first group are equipped with large number of straight slender microvilli, whereas those of the second group are characterized by occurrence of apical protrusions. In these cells, vacuoles are rarely found in supranuclear cytoplasm; however, dense osmiophilic granules are abundant in the whole cytoplasm. All nonciliated cells possess common ultrastructural characteristics (Goyal and Hrudka, 1980; 1981). The nuclei are less electron-dense than those of ciliated cells (Wrobel, 1972). They were euchromatic and located in the basal third of the cells (Goyal and Hrudka, 1980; 1981). A small Golgi apparatus is found juxtanuclear, endoplasmic reticulum is less developed and long mitochondria are found next to lateral cell membrane and in the infranuclear area (Wrobel, 1972; Goyal and Hrudka, 1980; 1981). The luminal surfaces of nonciliated cells possess either a uniform microvillous border or an alternation of microvillous border and apical protrusions of various sizes (Goyal and Hrudka, 1980; 1981). These protrusions may contain granules or vacuoles that are found released in the lumen along with granules and vacuoles. Nonciliated cells possess an endocytotic apparatus beneath their microvillous border (Wrobel, 1972; Goyal and Hrudka, 1980; 1981). The second type of nonciliated cells corresponded to type II cells described by Goyal and Hrudka (1980; 1981). They possess round, mostly homogenous and membrane-bound granules, which apparently fill the cytoplasm. The granules frequently present variation in shape, number, size and density among different cells or even within the same cell. They originate from expanded rough endoplasmic reticulum (rER). These findings are in accord with those mentioned by Goyal and Hrudka (1980; 1981). The third type of nonciliated cells was similar to type III cells reported by Goyal and Hrudka (1980; 1981). These cells are characterized by occurrence

of vacuoles in their supranuclear cytoplasm. The vacuoles demonstrate small flocculent and membranous content (Wrobel, 1972). This may indicate low density phospholipids (Goyal and Hrudka, 1980; 1981). Mitochondria are arranged in a row along the lateral cell membrane and presented typical transverse cristae. Sporadic profiles of rER were encountered, whereas Golgi cisternae enclosed pale contents similar to that of the vacuoles.

Ciliated cells are distinguished not only by the cilia but also by the apical position of their nuclei, which are ovoid and heterochromatic (Wrobel, 1972; Goyal and Hrudka, 1980; 1981). The supranuclear cytoplasm contains a small Golgi apparatus, few irregularly-shaped dense bodies and many mitochondria. The latter are abundant both in apical and basal regions. Similar findings were reported by Wrobel (1972) and Goyal and Hrudka (1980; 1981). Contrary to those of nonciliated cells, mitochondria possess fewer and mostly longitudinally oriented cristae. Endoplasmic reticulum is inconspicuous. The apical surfaces are equipped with typical kinocilia with distinct basal bodies as well as a few microvilli scattered in-between. Elements of the endocytotic apparatus are ill-developed.

The occurrence of basal cells in the ED is disputed. Whereas Wrobel (1972) found basal cells that were characterized by paucity of organelles, no basal cells were reported in the work of Goyal and Hrudka (1980, 1981). In agreement with my findings, Goyal and Hrudka (1980; 1981) reported the occurrence of intraepithelial lymphocytes (IEL) and intraepithelial macrophages (IEM). On the other hand, Wrobel (1972) did not refer to the intraepithelial leucocytes in bovine ED. I see that he mistook and considered the intraepithelial leucocytes as basal cells.

5.4.2 Epididymal duct

In most species the epididymal epithelium is made up mainly of two cell types namely principal and basal cells. Some scattered AMRC occur in certain regions of the epididymis as well as intraepithelial lymphocytes and macrophages. Principal cells (PC) constitute the vast majority of cells throughout the length of the duct in all species (Abe et al., 1983; Arrighi et al., 1986; 1993, Chandler et al., 1981; Flickinger et al., 1978; Hermo et al., 1991; Hoffer et al., 1973; Nicander and Plöen, 1979; Piomboni, 1997; Ramos and Dym, 1977a; Sinowatz, 1981). Regarding their size and content of organelles, PC exhibit characteristic differences in the different segments of the duct. These differences indicate special cellular activities in the different regions of the epididymal duct (Sinowatz, 1981).

The apical surfaces of PC possess stereocilia, which show variable length in the different segments of the bovine epididymal duct. Their length reaches about 18 μm in the first

segment and decreases gradually up to 6 µm in the sixth segment. They are long microvilli with a filamentous core (Sinowatz, 1981). They increase the area of the epithelial luminal surface and consequently the volume of the transport of luminal contents into the epithelium. Furthermore, they possess enzyme activities characteristic in the different segments of the duct and thus specific and variable resorptive activities (Sinowatz, 1981).

The ultrastructural characteristics of PC in the segments of caput (I, II, and III) and corpus (IV and V) epididymidis in bull reveal an unequivocal endocytotic activity. The PC in segment I possess numerous pinocytotic invaginations and vesicles between the bases of stereocilia and just beneath the apical cell membrane. Moreover, several smooth-surfaced and coated vesicles occupy approximately the apical half of the supranuclear zone of cytoplasm. In this segment, large numbers of small multivesicular bodies (MVBs) are found in the apical and supranuclear area of cytoplasm. The PC of segment II present abundance of vacuoles as well as some MVBs in their apical cytoplasm. The apical portions of the PC of segment III display plenty of pinocytotic invaginations, vesicles and vacuoles. The PC of segments IV and V contain smooth-surfaced and coated vesicles and occasionally large MVBs within their apical cytoplasm. Goyal (1985), on the other hand, found major differences between the PC of both segments, which include absence of large vacuoles and MVBs in segment V. The PC of the sixth segment are characterized by short stereocilia and few numbers of pinocytotic vesicles. Thus the resorptive process in cauda epididymidis is comparatively less than that of the other epididymal segments (Sinowatz, 1981). Similar findings were reported in the PC of the epididymal epithelium in rat (Hoffer et al., 1973), mouse (Abe et al., 1983), rabbit (Nicander and Plöen, 1979), hamster (Flickinger et al., 1978), cat (Arrighi et al., 1986), dog (Chandler et al., 1981), horse (Arrighi et al., 1993), monkey (Ramos and Dym, 1977 a) and human (Piomboni, 1997).

The apical zone of PC cytoplasm in the first three segments possesses an extensive Golgi apparatus as well as numerous profiles of sparsely granulated endoplasmic reticulum. The Golgi apparatus is always located in the juxtanuclear area. Its size is markedly reduced in the fourth and the fifth segments. In the sixth segment it increases again. Despite the extensive size of the Golgi apparatus in the proximal segments of the epididymal duct, comparatively few secretory granules could be observed in the supranuclear cytoplasm. Similar findings were reported in different species including cat, dog, pig, sheep, cattle and horse (Nicander and Malmqvist, 1977). Evidence for the secretion of glycoproteins was reported by early autoradiographic studies (Neutra and Leblond, 1966) and has been supported by ultra-structural studies using radioactive sugars and amino acids (Flickinger, 1979; 1985). Despite

evidence for synthesis and secretion of proteins, it has consistently been reported that these cells lack typical secretory granules (Hoffer et al., 1973; Hamilton, 1975; Flickinger, 1979). But in my opinion, I see that the Golgi apparatus is a crucial organelle for protein synthesis both for intra- and extracellular use. Consequently, in addition to its role in the secretion of certain glycoproteins for extracellular use, Golgi apparatus participates also in the synthesis of lysosomes, which are utilized for the intracellular digestion of the reabsorbed material (Flickinger et al., 1978; Sinowatz, 1981).

Numerous membrane-bound heteromorphic granules are found in the infranuclear and more frequently in the basal region of PC along the length of the duct. Similar granules were reported by Sinowatz (1981) who suggested that these granules are presumably derived from Golgi apparatus. Histochemical studies (Sinowatz, 1981) showed that these basally located granules contain high concentration of hydrolytic enzymes. Furthermore, these granules exhibit variable histochemical and ultrastructural characteristics in different segments of the duct (Sinowatz, 1981). Apart from its role in the synthesis of lysosomal and secretory glycoproteins, the Golgi apparatus may participate in the synthesis of integral membrane proteins (Sinowatz, 1981). A significant membrane turnover of the luminal plasmalemma is associated with extensive endocytotic activities and may be one of the important tasks of the Golgi apparatus (Moore and Bedford, 1979).

The apical zones of the PC of caput and corpus epididymidis possess plenty of short profiles of endoplasmic reticulum (ER). The latter is mainly of the smooth type and sometimes is sparsely granulated. Similar findings were recorded in rat (Hoffer et al., 1973; Hermo et al., 1991), mouse (Abe et al., 1983), guinea pig (Hoffer and Karnovsky, 1981), hamster (Flickinger et al., 1978), cat (Arrighi et al., 1986), bull (Sinowatz, 1981), stallion (López et al., 1989) and monkey (Ramos and Dym, 1977a). In addition to short profiles of endoplasmic reticulum, large numbers of rER in form of long cisternae is found around the Golgi complex and near to the lateral cell membrane in the first three segments. Similar findings were recorded in rat (Hoffer et al., 1973), mouse (Abe et al., 1983), hamster (Flickinger et al., 1978), cat (Arrighi et al., 1986), bull (Sinowatz, 1981; Goyal, 1985) and human (Piomboni, 1997). In accord with Sinowatz (1981), rER of the PC in segments IV and V, is found immediately above the nucleus, whereas in segment VI it occurs in the middle zone of the supranuclear area. Moreover, the topographic relationships of cisternae of the ER (site of protein biosynthesis) with the Golgi apparatus (site of terminal glycosylation and packaging of proteins), have been described in previous electron microscopic studies (Farquhar and Palade, 1981; Sinowatz, 1981).

Apical protrusions are regularly found in PC of the second epididymal segment, even at the light histological level. Furthermore, Agrawal and Vanha-Perttula (1988) recorded that apical protrusions are very frequent findings in the PC of the bovine epididymis and suggested that these structures take part in the apocrine secretory mechanism.

Mitochondria of PC show considerable variation regarding their forms, internal structure and intracellular distribution throughout the different segments of the bovine epididymal duct. Moreover, the activity of the mitochondrial dehydrogenases shows also some variation along the length of the duct (Sinowatz, 1981). Mitochondria are generally concentrated in the infranuclear, and to lesser extent, in the basal area of the PC. Some scattered mitochondria are also encountered in the supranuclear area of the cytoplasm of the PC in the segments III-V. A large aggregation of mitochondria is found directly above the nuclei of the PC of the segment VI. In agreement with the findings recorded by Sinowatz (1981), the form and fine structure of mitochondria show remarkable variation between the different segments. Similarly, mitochondria of the PC of the cat epididymis were heterogeneous (Arrighi et al., 1986). Furthermore, there is a correlation between the high content of the mitochondria in PC of the epididymal epithelium and the building up of acidic secretory products and participation in an osmotic pump (Ramos and Dym, 1977a). Moreovere, Holstein (1969) proposed that the mitochondria may stimulate the synthetic activity of rER, as he found a close contact between mitochondria and rER in the human epididymis.

The nuclei of the PC show a marked variation in their form, morphological characteristics and their location within the epithelium along the length of the epididymal duct. The nuclei of the first segment are oval in shape and located in the basal third of the cells. They possess moderate content of heterochromatin which includes a narrow peripheral rim as well as small islands of heterochromatin throughout the karyoplasm. Many of them have one or more compact nucleoli. Apart from their ovoid to spherical form and shorter dimension, the nuclei of the PC of the second segment are similar to that of the first segment. The bizarre contour of the nuclei is a characteristic feature for the PC of the third segment (Sinowatz, 1981). Nuclei of this segment appear often subdivided in chromatin strands, which were bound together via narrow bridges and many of them possess basket-shaped nucleoli. The nuclei of segments IV and V are elongated and often contain one or more honeycomb-like nucleoli. The nuclei of the sixth segment demonstrate a profile similar to that of the third segment regarding their irregular contour. Similar findings were reported in monkey (Ramos and Dym, 1977a) epididymis where the nuclei of the PC were progressively more infolded in the regions of distal head, body and particularly in the tail region where their extreme lobation is a very

characteristic feature for this region. The infoldings in the nuclear membrane may correlate to higher metabolic and synthetic activities in these segments (Ramos and Dym, 1977a).

Whereas the PC exhibit clear morphological differences along the length of the epididymal duct, the basal cells (BC) exhibit a rather uniform morphology throughout the entire length of the duct. They possess a moderate content of organelles including mitochondria, inconspicuous Golgi apparatus, few cisternae of ER and free ribosomes as well as bundles of cytoplasmic filaments which are oriented parallel to basal membrane. Lipid droplets are frequent, but their number and size is smaller in proximal than in distal segments of the duct. A close relationship between mitochondria and lipid droplets is noticeable. Similar findings were recorded to the BC in the epididymis of hamster (Flickinger et al., 1978), cat (Arrighi et al., 1986), dog (Schimming and Vicentini, 2001), horse (Arrighi et al., 1993), and monkey (Ramos and Dym, 1977a).

In addition to PC and BC, apical mitochondria-rich cells (AMRC) were also encountered in the bovine epididymal epithelium. These cells are comparatively frequent in the second and the third segments and are found only rarely in other segments of the duct (Sinowatz, 1981; Goyal, 1985). They are characterized by their ovoid nuclei, which lie in the apical third of the epithelium (Sinowatz, 1981). With the exception of a fair number of mitochondria and free ribosomes, they are characterized by their scarcity of organelles (Sinowatz, 1981; Goyal, 1985). Moreover, they are encountered in both segments (II and III) where mitotic figures are not infrequent and they possibly represent transitional cell stage in their way to replace degenerated PC (Sinowatz, 1981). Similar findings were also recorded in the epididymal epithelium of different species including rat (Hoffer et al., 1973), hamster (Flickinger et al., 1978), cat (Arrighi et al., 1986), dog (Schimming and Vicentini, 2001), goat (Goyal and Williams, 1991), horse (Arrighi et al., 1993), monkey (Ramos and Dym, 1977a) and human (Palacios et al., 1991). AMRC possess microvilli which are shorter than stereocilia of the PC. They contain numerous electron-dense granules and vacuoles showing a variable size and content in the apical cytoplasm. Similar findings were reported in different mammalian species (Martínez-Garcia et al., 1995). The most characteristic feature of these cells is the abundance of mitochondria (Abou-Haila et al., 1985; Abou-Haila and Fain-Maurel, 1984; Goyal, 1985; Martínez-Garcia et al., 1995; Palacios et al., 1991; Ramos and Dym, 1977a; Sun and Flickinger, 1980; Sinowatz, 1981). Their mitochondria vary in size and shape but are mainly located in the supranuclear cytoplasm and around the nucleus (Martínez-Garcia et al., 1995). The hyaloplasm of AMRC is lighter in bull (Goyal, 1985) and darker in human

(Palacios et al., 1991) than that of the adjacent PC, whereas in the mouse (Abou-Haila et al., 1985) the hyaloplasm of both cell types show a similar electron density.

Intraepithelial or migratory leucocytes are first described by Reid and Cleland (1957) as halo cells. Hoffer and co-workers (1973) described these cells as agranular leukocytes and reported that many of them are not typical migratory lymphocytes. However, Hamilton (1972) recorded that the halo cells possess many ultrastructural characteristics of lymphocytes. Similar findings were reported by Flickinger and co-workers (1978) in the epididymal epithelium of the hamster. Intraepithelial lymphocytes (IEL) were also found throughout the entire length of the bovine epididymal duct (Sinowatz, 1981; Goyal, 1985). Despite occurrence of large numbers of IEL in the basal area, they can be encountered at different levels of the epithelium (Sinowatz, 1981). The same author reported that they are often found in an immediate proximity with the subepithelial capillaries. My findings are in agreement with those reported by Sinowatz (1981). Their ultrastructural characteristics are round, very heterochromatic and indented nuclei as well as a cytoplasm that is lighter than that of neighbouring cells (Sinowatz, 1981; Goyal, 1985). It is characterized by paucity of organelles (Sinowatz, 1981). IEL were also found in the epididymal epithelium of other species including rat (Hamilton, 1972; Hoffer et al., 1973), hamster (Flickinger et al., 1978), cat (Sanchez et al., 1998), goat (Goyal and Williams, 1991), horse (López et al., 1989; Arrighi et al., 1993), monkey (Ramos and Dym, 1977a, 1978) and human (Piomboni, 1997).

In contrast to lymphocytes, intraepithelial macrophages (IEM) are only very rarely seen at higher levels of epididymal epithelium. They can be observed mainly in the basal area of the epithelium and contained numerous acid phosphatase-positive globules (Sinowatz, 1981). IEM are characterized by their pseudopodic cytoplasmic processes lodged between PC and BC. The nucleus is small, heterochromatic and is surrounded by many mitochondria, well-developed Golgi apparatus and rER. Various profiles of lysosomes as well as dense globules are found peripherally (Sinowatz, 1981). Similar findings were recorded in the epididymal epithelium of cat (Sanchez et al., 1998), goat (Goyal and Williams, 1991) and human (Piomboni, 1997).

The epididymal epithelium is separated from the underlying structures by a well-developed basal lamina (BL). BC are connected to the BL by a large number of hemidesmosomes. Underneath the BL a thin layer of connective tissue consisting of fibroblasts and collagen fibrils is longitudinally oriented. There is usually a definite line of demarcation between the collagen fibrils and SMC. The amount of the latter increases continuously and reaches its maximum width in the sixth segment. The individual SMC show only minimal variation in

their form throughout the length of the duct. Similar findings were recorded by Sinowatz (1981) and Goyal (1985). The interstitial space is occupied by loose connective tissue with the blood and lymph vessels as well as nerves. Fibroblasts, macrophages, granulocytes, lymphocytes and mast cells constitute together the cellular elements of the interstitial tissue (Sinowatz, 1981; Goyal, 1985). Similar findings were recorded for the dog epididymis (Chandler et al., 1981).

In mammalian epididymis, peritubular muscle coat (PMC), elaborate tight junction and desmosomes serve as a barrier between the luminal fluid and blood capillaries (Agarwal and Hoffer, 1989). The epididymal tight junctions are highly developed when compared with the other epithelial cells. They form a continuous zone around the cell, sealing the spaces between the epithelial cells so that the luminal space and the intercellular spaces become separate physiological compartments (Friend and Gilula, 1972). In the epididymis, the zonulae occludentes near the luminal surface are exclusively responsible for the maintenance of the blood-epididymis barrier, since neither the capillary endothelium nor the PMC significantly impedes the flow of the tracers towards the epididymal lumen (Hoffer and Hinton, 1984).

In accord with my findings, junctional complexes are well-developed between the adjacent plasma membrane of the apical portions of the PC in the epididymis of cat (Arrighi et al., 1986), dog (Chandler et al., 1981), bull (Sinowatz, 1981; Goyal, 1985) and monkey (Ramos and Dym, 1977a).

5.5 Functional correlates

5.5.1 Efferent ductules

According to Carbo (1965), most of the rete testis fluid is reabsorbed by ED of bull and boar. Levin and Marsh (1971) come to a similar conclusion in rat. The resorptive activity of bovine ED is indicated by the uptake of the protein tracer (horseradish peroxidase) and the presence of microvilli with alkaline phosphatase activity (Goyal and Hrudka, 1980; 1981). The same authors also recorded that all nonciliated cells possess resorptive activity as indicated by existence of a well-developed endocytotic apparatus represented by elaborative canalicular system, pinocytotic vesicles, coated vesicles and subapical vacuoles. Similar findings are recorded also in ED of bull (Wrobel, 1972), goat (Goyal and Williams, 1988; Goyal et al., 1992), stallion (López et al., 1989; Arrighi et al., 1993), macaque monkey (Ramos and Dym, 1977b), hamster (Flickinger et al., 1978), and rat (Hermo and Morales, 1984).

There is ample evidence that bovine ED also engage in secretory activity. This is indicated by provision of secretory machinery (rER, Golgi apparatus, mitochondria, and specific granules and vacuoles) and dynamics of elaboration of secretory granules or vacuoles, their storage and release into the lumen (Goyal and Hrudka, 1981). On the other hand, Ramos and Dym (1977b) claimed that the protein synthesis and secretion is not a major function of the epithelium of monkey ED. The authors ascribed their interpretation to the paucity of organelles like endoplasmic reticulum and Golgi apparatus. Apical protrusions in the epithelium of human ED were taken as a sign of secretory activity (Morita, 1966; Holstein, 1969). A similar proposal was given by Goyal and Hrudka (1981), who exclude the possibility that apical protrusions are related to the absorptive process as assumed by Wrobel (1972). Persistence and abundance of granules in nonciliated cells after ligation of goat ED (deprivation of the epithelium of rete testis fluid) excluded the notion of their absorptive nature (Gray et al., 1983). The same authors, in disagreement with Hamilton (1975), mentioned that these granules are not of lysosomal nature and suggest that they are secretory. Goyal and Hrudka (1981) suggested that the conspicuous existence of granules in bovine ED may indicate their important role in sperm maturation. These specific granules may be of significance in providing sperm antigens (Barker and Amman, 1971) or sperm coating proteins (Lea et al., 1978). They contain glycoprotein which could be a source of nutrients and/or other factors necessary for priming sperm motility (Blandau and Rumery, 1964; Brandt et al., 1978). The role of vacuoles in the ED remains unclear. They presumably are of some significance in acidification of luminal fluid, which is believed to take place in this region of the male excurrent ducts (Levin and Marsh, 1971).

5.5.2 Epididymal duct

It is evident that the epithelium of the epididymis is both absorptive and secretory. Furthermore, the same (principal) cell may subserve both functions (Ramos and Dym, 1977a; Moore and Bedford, 1979b). The epididymal fluid has been reported to be regionally different in chemical composition and this difference is ascribed to the variable functional activity of the epithelium in the different segments (Levin and Marsh, 1971; Abe et al., 1983). Whereas the PC of the proximal (I, II and III) segments of the mouse epididymis exhibited both absorptive and secretory ultrastructural characteristics, those of the distal (IV and V) segments are mainly absorptive (Abe et al., 1983). Though the existence of a well-developed Golgi apparatus and abundant rER indicates secretory function of the PC, they also possess absorptive activity as indicated by pinocytotic uptake of horseradish peroxidase (Moore and

Bedford, 1979b). Testicular fluid is reabsorbed in the ED and caput epididymidis causing sperm cell concentration to increase along the epididymis. Most proteins of rete testis fluid could not be found in the luminal fluid of caput epididymidis as a result of a probable selective resorption by the lining epithelium of ED and proximal epididymal segments (Dacheux et al., 1989). Thus the protein composition of luminal fluid changes drastically between the testis and the epididymis. Although most proteins of rete testis fluid are endocytosed in the ED, the microinjection studies revealed that the testicular fluid is taken up in a limited extent in the caput region (Veeramachaneni et al., 1990). Androgen-binding protein (ABP) is selectively spared and concentrated for possible use at more distal epididymal sites (Veeramachaneni et al., 1990) in the caput region (Attramadal et al., 1981; Gerard et al., 1988) where 5α-reductase activity is greatest (Veeramachaneni et al., 1990). It is well established that the epididymis is an androgen-dependent organ (Arya and Vanha-Perttula, 1985b). Androgens reach the epididymis either via circulation or in the testicular fluid attached to ABP. They perform their biological response in the epididymal target cells by binding to specific cytoplasmic and nuclear androgen receptors (Tezon and Blaquier, 1983).

It has been postulated that the mechanisms involved in the absorption of testicular fluid is a process of pinocytosis (Burgos, 1964; Glover and Nicander, 1971; Hoffer et al., 1973; Ramos and Dym, 1977a; Flickinger et al., 1978; Moore and Bedford, 1979b; Goyal and Hrudka, 1980; Chandler et al., 1981; Sinowatz, 1981; Abe et al., 1983; Djakiew et al., 1984; Hermo and Morales, 1984, Goyal, 1985; López et al., 1989). This mechanism is quantitatively sufficient to account for the fluid reabsorbed in the proximal region of the epididymis when correlated with the consideration of the great length of the epididymal duct and the limited daily volume of the fluid uptake (Hoffer et al., 1973). The presence of abundant vesicles and vacuoles in the epithelium of the male excurrent ducts of hamster (Flickinger et al., 1978) was correlated with the sites of fluid resorption. However, the abundance of vesicles and vacuoles decreased further along the length of the duct (Flickinger et al., 1978) as proved by micropuncture studies (Jessee and Howards, 1976). On the other hand, Glover and Nicander (1971) reported some resorption in the initial and terminal segments of mammalian epididymis as indicated by micropinocytosis, apical vesicles and MVBs. However, conspicuous pinocytosis as well as distinct supranuclear vacuoles were common morphological characteristics of the PC in the first portion of the middle segment reflecting massive resorption of the fluid in this region as demonstrated by obvious crowding of

spermatozoa in the second portion of the same segment. The PC of the equine caput and corpus epididymidis are equipped with long stereocilia and display morphological hallmarks of absorption and digestive activities (López et al., 1989). Similar findings were recorded in the epididymis of mouse (Abe et al., 1983), dog (Chandler et al., 1981), bull (Sinowatz, 1981; Goyal, 1985) and monkey (Ramos and Dym, 1977a).

It is well established that sperm maturation is not intrinsic to sperm cells themselves but requires their interaction with proteins that are synthesized and secreted by the epididymal epithelium (Cornwall and Hann, 1995). Studies concerned with gene expression indicated that the caput epididymidis is a very active region in protein synthesis and secretion (Cornwall and Hann, 1995). Similarly, Kohane and colleagues (1980) recorded that the rat epididymis produces and secretes specific proteins, which interact with the maturing spermatozoa and are involved in their maturation process. Immunolocalization of these proteins indicated that the PC of the caput epididymidis are the main site of protein synthesis. Moreover, concentration of protein in luminal fluid progressively increases in the caput epididymidis and attains its highest values in distal caput of bull and ram (Carbo, 1965; Veeramachaneni et al., 1990). On the other hand, Pavlović and co-workers (1981) reported in their immunohistochemical studies that the epithelium of the bovine cauda epididymidis possesses greater secretory activity than that of the caput. Despite the absence of classical secretory granules, existence of a well-developed Golgi apparatus and abundant rER in the PC of the epididymal epithelium (Hoffer et al., 1973; Nicander and Malmqvist, 1977; Liu et al., 1991) is suggestive of active glycoprotein synthesis. This is in accordance with the findings in the PC of monkey (Ramos and Dym, 1977a) and horse (López et al., 1989; Arrighi et al., 1993) epididymis. Furthermore, the elaborate infoldings of the nuclear membrane and prominent nucleoli might reflect a highly intense metabolic activity (Ramos and Dym, 1977a; López et al., 1989). *In vitro* studies reported that epididymal epithelial cells in culture secrete proteins for many days (Moore et al., 1992; Akhondi and Moore, 1993) in the presence of androgens. This lends support to the notion of Arya and Vanha-Perttula (1985b) that androgens activate the biosynthetic machinery (Tezon and Blaquier, 1983) of the PC resulting in the production and secretion of multiple glycosylated proteins. Similarly, Faye and colleagues (1980) stated that sialoproteins are produced and secreted in an androgen-dependent manner by the PC lining the caput and corpus epididymidis of rat. Furthermore, sialoproteins exhibit characteristics very similar to those of acid epididymal glycoprotein (AEG) described by Lea and co-workers (1978). Some of the secretory glycoproteins in the epididymal fluid possess a well established

role in the induction of forward motility (Brandt et al., 1978; Acott and Hoskins, 1981) and acquisition of fertilizing capacity (Lea et al., 1978; Orgebin-Crist and Jahad, 1978; Faye et al., 1980; Arya and Vanha-Perttula, 1985b). Moreover, alterations of sperm membranes result from the incorporation of proteins, sugars and lipids which are of epididymal origin (Moore, 1998). Furthermore, spermatozoon antigens can be synthesized by PC and secreted into the epididymal fluid. They are bound on the surface of spermatozoa either through direct covalent binding, via transferases or by direct membrane interchange (Moore, 1998).

In contrast to PC, BC appear quite inactive as demonstrated by paucity of organelles (Sinowatz, 1981). The function of the BC is to great extent unknown. Amann (1989) reported that the BC do not perform a specific function. They probably perform a supportive function and confer stability to epididymal epithelium by means of their high content of cytoplasmic filaments, interdigitation with adjacent PC and anchoring with basal membrane via numerous hemidesmosomes (Holstein, 1969; Hamilton, 1975; Ramos and Dym, 1977a). Although Sinowatz (1981) recorded similar characteristics in the BC of bovine epididymis, he excludes a supportive role of the BC because they have only a very small contact area with the basal membrane. Other authors assume that BC are reserve cells (Bidwai and Bawa, 1981) for epididymal epithelium renewal. However, this notion was disproved by Sinowatz (1981) and Arrighi and co-workers (1993) who did not find any mitotic activity in the basal epithelial area of the bovine and equine epididymis respectively. In the present work, BC expressed strong FGF-2-immunoreaction along the entire length of the epididymal duct, thus they may play a role in induction of proliferation, growth and differentiation of the PC at least in the bovine epididymis via a paracrine mechanism (Bikfalvi et al., 1997b).

Although the exact functional significance of the apical mitochondria-rich cells (AMRC) is not yet known (Martínez-Garcia et al., 1995), several functions have been suggested. They could be involved in resorption (Sun and Flickinger, 1980) and acidification (Levine and Kelly, 1978; Au and Wong, 1980) of the epididymal fluid. AMRC of the rat and the golden hamster contain numerous apical vacuoles that suggest active endocytosis (Cooper et al., 1988). Furthermore, Hermo and Morales (1984) reported that ultrastructural characteristics of these cells indicate their involvement in the uptake and eventual degradation of internalized proteins. Carbonic anhydrase has been localized in AMRC in the rat (Cohen et al., 1976; Sun and Flickinger, 1980) and it has been suggested they are involved in acidification (Jensen et al., 1999) of the epididymal fluid through the secretion of protons (Cohen et al., 1976) as a

result of bicarbonate resorption (Jensen et al., 1999). On the other hand, Adamali and Hermo (1996) reported that AMRC in the rat epididymis were unreactive for carbonic anhydrase. In the present work AMRC were negative for ACE and S-100 proteins, which are involved in the regulation of fluid and electrolyte transport in the epididymis. ACE can induce local generation of angiotensin II (O'Mahony et al., 2000) in the epididymis. Angiotensin II stimulates the anion transport of the epididymal epithelium. In accordance with Adamali and Hermo (1996), who found no reactivity for carbonic anhydrase and from my findings, I assume that AMRC play no significant role in regulation of fluid and electrolyte movement in the bovine epididymis.

Physiological and morphological studies showed that the principal component of a given blood tissue barrier differs from an organ to another (Fawcett et al., 1970; Hoffer and Hinton, 1984). Zonulae occludentes are the principal component of the blood-epididymis barrier (Hoffer and Hinton, 1984; López et al., 1997). Spermatozoa which mature and are stored in the epididymis are immunogenic (Hinton et al., 1996). Therefore, epididymis has to provide mechanisms protecting spermatozoa from autoimmune reactions (Marchlewicz, 2001). The antigenic material sequestration (Marchlewicz, 2001) in the normal epididymis is offered through two principal possibilities:

- Apically located tight junctions between plasma membranes of the adjacent PC do not permit the passage of spermatozoa or their antigens from the epididymal lumen into the blood vessels.
- Immune tolerance offered by intraepithelial leucocytes.

Autoimmune reactions to spermatozoa may arise against some molecules acquired by later stages of male germ cells, which are not expressed until after puberty and therefore are not identified as self antigens by the immune system, early in the life (Tung and Menge, 1985). These immunogenic components on these germ cells are physically sequestrated from the immune system by the blood-testis barrier (Dym and Fawcett, 1970) and by the blood-epididymis barrier (Hoffer and Hinton, 1984). Immune privilege appears to be a physiological strategy designed to prevent immunopathogenic processes from disrupting the critical functions of particularly vulnerable organs and tissues (Streilein, 1993). It is an active process and its creation results from local tissue factors that interact with and alter the functional programs of the immune system. Many privileged sites share certain common features, such as the presence of immunosuppressive cytokines and factors that can suppress antigen-driven T-cell activation (Streilein, 1993). Furthermore, it is evident that there are local immunosuppressive mechanisms (Mahi-Brown et al., 1988; Pöllänen and Setchell, 1990) in the male

reproductive tract. Although, it has been reported that the majority of lymphocytes found in the normal human testis and epididymis are suppressor/cytotoxic $CD8^+$ T-cells (El-Demiry et al., 1985), these functions are no longer associated strictly with $CD8^+$ T-cells (Mahi-Brown et al., 1988). Furthermore, since any T-lymphocyte can function as a suppressor under appropriate conditions (Melchers, 1987), the murine $CD4^+$ T-cells which were previously considered as helper/inducer cells, could be cytotoxic to targets bearing class II MHC molecules (Itoh et al., 1992). Therefore, $CD4^+$ T-cells may play a role in suppressing autoimmune responses to sperm antigens that normally leak from the epididymal duct (Flickinger et al., 1997). Furthermore, both $CD4^+$ and $CD8^+$ T-cell populations may be important regulatory elements governing the suppression of testicular autoimmunity in mice and there must be different suppressive mechanisms between $CD4^+$ and $CD8^+$ T-cells (Itoh et al., 1992).

Mast cells (MC) are multifunctional long-lived cells. They are characterized by its content of numerous large cytoplasmic granules. They develop from the haematopoietic stem cells. MC leave the bone marrow and circulate as immature committed progenitors (Gurish and Austen, 2001). The circulating committed progenitors are agranular mononuclear cells, which traverse the vascular space and complete their maturation after moving into diverse peripheral tissues (Crivellato et al., 2004). Local differentiation and maturation of MC are most likely controlled by tissue microenvironment factors, particularly stem cell factor (SCF) secreted by stromal cells, fibroblasts and endothelial cells, which represents the most important cytokine involved in MC development. Survival and maturation of tissue MC are also enhanced by other cytokines including interleukins (Conti et al., 2002). MC are particularly prominent in close vicinity to blood and lymphatic vessels under physiological conditions. In addition to their effector roles in the induction of IgE-associated allergic disorders, MC play important roles in host defence against invaders. Moreover, they exert distinct non-immunological functions, playing a relevant role in tissue homeostasis, remodelling and fibrosis as well as angiogenesis (Artuc et al., 2002). MC release a variety of angiogenic factors (Crivellato et al., 2004). MC can synthesize and secrete VPF/VEGF. Moreover, FGF-2 and VEGF are immunolocalized in the mature tissue MC (Grützkau et al., 1998). Thus they regulate angiogenesis and vascular permeability as well as many other VPF/VEGF-mediated biological activities (Boesiger et al., 1998).

6 Summary

Glycohistochemical, Immunohistochemical and Ultrastructural Studies of the Bovine Epididymis

In the present work, efferent ductules and epididymal duct from male foetuses as well as from sexually mature bulls were investigated using conventional light and electron microscopical techniques as well as glycohistochemical and immunohistochemical staining techniques. The prenatal development of the bovine epididymis was studied in foetuses ranging from 10 cm CRL (75 pcd) to 90 cm CRL (285 pcd). In foetuses with 10 cm CRL (75 pcd) the main event was the establishment of the urogenital junction between the extratesticular rete testis and mesonephric duct via the growing efferent ductules. At the foetal age of 110 pcd (24 cm CRL), efferent ductules underwent a strong coiling. At the same time the mesonephric duct began to lengthen and coil, forming three distinct regions, namely caput, corpus and cauda epididymidis. The coiling was much more distinct in caput and cauda than in corpus epididymidis. At 130 pcd (30 cm CRL) and upwards efferent ductules were organized in lobules which are then arranged in groups separated from each other by connective tissue septa. A similar organization involved the highly convoluted epididymal duct, particularly in the head and tail regions. In addition to the macroscopical modifications in the morphology of extratesticular excurrent duct system, histological differentiation involved both the tubular epithelium and the peritubular mesenchymal cells. The epithelium of efferent ductules was differentiated into ciliated and nonciliated columnar epithelium. The simple epithelium of the epididymal duct increased in height and developed stereocilia on its apical surface. Distribution of WGA-, PNA- and GSA-I-binding sites on luminal surface of the epithelium of efferent ductules, but not of epididymal duct may indicate earlier differentiation of the former. WGA-binding to the peritubular and interstitial mesenchymal cells, but not to the epididymal epithelium indicated that the mesenchymal structures differentiate before epithelial ones.

S-100, FGF-1, FGF-2, ACE, laminin and GT were immunolocalized in the epithelium both of efferent ductules and epididymal duct as early as at 75 pcd (10 cm CRL). Also α-SMA was immunolocalized in the peritubular mesenchymal cells at 75 pcd (efferent ductules) and at 95 pcd (epididymal duct, CRL 18 cm).

The epithelium of the adult bovine efferent ductules is simple columnar including ciliated and nonciliated cells as well as some scattered intraepithelial leucocytes. On the basis of their cytological characteristics, nonciliated cells could be categorized into three sub-types.

The epididymal duct of the adult bull is lined with pseudostratified columnar epithelium. It consists mainly of tall, slender, stereocilia-bearing columnar cells and small basal cells. On the basis of several morphometric parameters like epithelial height, luminal diameter and width of peritubular muscle coat the epididymal duct could be subdivided into six segments. Ultrastructural studies revealed a well developed Golgi apparatus, numerous profiles of sparsely granulated endoplasmic reticulum and mitochondria as well as rER in the cytoplasm of principal cells particularly in those of the first three segments. Apical surfaces of principal cells particularly those of the proximal segments were equipped with long stereocilia and their apical cell membrane and cytoplasm displayed a well developed endocytotic apparatus. The narrow basal extensions of principal cells were crowded with numerous pleomorphic mitochondria, lysosomes, heteromorphic electron dense granules and residual bodies. Basal cells were insinuated between the narrow basal extensions of principal cells and the basal lamina. They possessed kidney-shaped, mostly deeply-invaginated nuclei and were characterized by a paucity of organelles. Apical mitochondria-rich cells were frequently found in segments II and III and rarely in segments IV and V. Their hyaloplasm was lighter than that of the neighbouring principal cells and their apical surfaces were provided with short microvilli. Apart from a reasonable number of mitochondria, small Golgi apparatus and sporadic strands of rER, they displayed a paucity of organelles. Intraepithelial macrophages were occasionally encountered in the basal third of the epithelium. They possessed many mitochondria, well developed Golgi apparatus and rER as well as small heterochromatic nuclei. Various profiles of lysosomes and dark residual bodies were found in their cytoplasm. Intraepithelial lymphocytes were characterized by their heterochromatic, round and mostly indented nuclei and narrow peripheral cytoplasmic rim. They were often encountered in immediate proximity to subepithelial capillaries.

Fluoresceinisothiocyanate (FITC)-labelled lectins (GSA-I, PNA, ECA, WGA, Con A, LCA, PSA, DBA, HPA, SBA, VVA, LTA and UEA-I) were also used for the study of the regional distribution of saccharide groups in adult bovine epididymal tissues. WGA, Con A, LCA, PSA, DBA and HPA bound distinctly to stereocilia of principal cells in the different segments. However, DBA- and HPA-binding sites were confined to stereocilia in caput region. WGA, LCA, PSA, DBA and HPA possessed distinct binding sites in Golgi zone of principal cells, mostly of the caput epididymidis. Basal cells reacted distinctly with WGA, Con A, LCA, PSA and HPA. Intraepithelial leucocytes displayed moderate binding sites for PNA, WGA, LCA and PSA. The basal membrane reacted moderately only with WGA.

Epididymal connective tissue showed weak to moderate binding only with ECA and WGA. GSA-I bound distinctly to vascular endothelium and could be applied as a good marker for bovine endothelium. Sperm cell mass bound WGA and PNA distinctly. No binding sites could be found for VVA, LTA or UEA-I.

Immunohistochemical studies used the Avidin-Biotin-peroxidase Complex (ABC) method for localization of S-100, FGF-1, FGF-2, ACE, GT, VEGF, α-SMA, laminin, connexin 43, CD4, CD8 and CD68 in the epididymis. The epithelium of the efferent ductules showed intense immunoreaction for S-100, FGF-1 and FGF-2 and a moderate immunostaining for ACE and GT. Principal cells of the first three epididymal segments exhibited a distinct immunostaining for S-100. They also showed a distinct immunoreactivity for FGF-1 throughout the different segments. Principal cells in the first, second and sixth segment displayed intense immunostaining for ACE. Immunostaining for GT in Golgi zone of the principal cells was intense (segments II and III), distinct (segments IV and V) and moderate (segments I and VI). Basal cells showed moderate (FGF-1) or intense (FGF-2) immunostaining in different epididymal segments. Intense immunostaining for ACE, laminin and α-SMA was found respectively in the endothelium, endothelial basal lamina and smooth muscle cells of blood vessels. The basal lamina of the epithelium and the peritubular smooth muscle cells displayed a moderate immunoreactivity for laminin. The peritubular smooth muscle cells manifested an intense immunostaining for α-SMA. $CD4^+$ T cells and $CD68^+$ macrophages were found within the epithelium and in the interstitium. Mast cells were conventionally stained with Alcian blue and Toluidin blue. They also displayed a distinct immunostaining for VEGF and FGF-2.

In conclusion, my study supports the previously proposed 6-segment scheme of bovine epididymis. Moreover, lectin histochemistry and immunohistochemistry were not only helpful tools in emphasising this scheme but also in correlating specific functional activities to certain regions. Lectins- and GT-binding sites as well as ultrastructural characteristics point to high synthetic and secretory activities of principal cells in the first three segments, as indicated by the well developed Golgi apparatus. Ultrastructurally, principal cells of the proximal three epididymal segments displayed a well developed endocytotic apparatus. This was reinforced by intense immunostaining for ACE in this region, which reflects extensive absorptive activities in this region. Existence of mast cells in the epididymal interstitium and T-lymphocytes and macrophages in the interstitium and within the epithelium may reflect their harmonized co-operation in the induction of immune tolerance in the bovine epididymis.

ZUSAMMENFASSUNG

Glykohistochemische, immunohistochemische und ultrastrukturelle Studien am Nebenhoden des Rindes (*Bos taurus*)

In der vorliegenden Arbeit wurden die Ductuli efferentes und der Ductus epididymidis männlicher Föten sowie geschlechtsreifer Stiere mit konventioneller Licht- und Elektronmikroskopie sowie mit glykohistochemischen und immunohistochemischen Techniken untersucht. Die pränatale Entwicklung des Rindernebenhodens wurde bei Föten der Scheitel-Steiß Länge (SSL) 10 cm (Tag 75 p.c.) bis 90 cm(Tag 285 p.c.) untersucht. Bei den Föten mit 10 cm SSL (Tag 75 p.c.) war die Etablierung der Kontaktzone zwischen dem extratestikulären Rete testis und dem Wolff´schen Gang über die wachsenden Ductuli efferentes gut erkennbar. Am 110. Tag p.c. (24 cm SSL) zeigen die Ductuli efferentes eine starke Schlängelung. Zu diesem Zeitpunkt nimmt der Wolff´sche Gang stark an Länge zu, knäuelt sich auf und bildete drei makroskopisch eindeutig identifizierbare Regionen, nämlich Caput, Corpus und Cauda epididymidis.

Ab Tag 130 p.c. (30 cm SSL) organisierten sich die Ductuli efferentes in Läppchen, die sich dann in Gruppen anordnen und durch Bindegewebe von einander getrennt werden. Eine ähnliche Organisation zeigt auch der stark gewundene Ductus epididymidis, besonders in der Kopf- und Schwanzregionen. Zusätzlich zu den bereits makroskopisch erkennbaren Änderungen in der Morphologie der extratestikulären samenleitenden Wege, kommt es zur histologischen Differenzierung des Epithels und der peritubulären mesenchymalen Zellen. Das Epithel der Ductuli efferentes differenziert sich in zilientragende Zellen und Hauptzellen. Das einschichtige isoprismatische Epithel des Nebenhodens wird höher und entwickelt Stereozilien auf seiner apikalen Oberfläche.

Mit lektinhistochemien Methoden wurde die Verteilung von Zuckerstrukturen im Nebenhoden untersucht. WGA-, PNA- und GSA-I-Bindungsstellen traten auf der luminalen Oberfläche des Epithels der Ductuli efferentes, aber nicht im Epithel des Nebenhodenganges auf. Dies deutet darauf hin, dass die Ductuli efferentes sich zeitlich vor dem Nebenhodenepithel differenzieren. Die WGA-Bindung an die peritubulären und interstitiellen mesenchymalen Zellen, und die fehlende Reaktion mit dem epididymalen Epithel zeigte an, dass die mesenchymalen Strukturen sich vor dem Epithel entwickeln.

Mit Hilfe von immunohistochemischen Methoden wurde die Verteilung von S-100, FGF-1, FGF-2, ACE, Laminin und GT im Nebenhoden untersucht. Alle Antigene ließen sich im Epithel der Ductuli efferentes und des Nebenhodens bereits ab Tag 75 p.c. (10 cm SSL) nachweisen. α-SMA war in den peritubulären mesenchymal Zellen schon am Tag 75 p.c. (Ductuli efferentes) bzw. am Tag 95 p.c. (SSL 18 cm, Ductus epididymidis) histochemisch nachweisbar.

Das Epithel der Ductuli efferentes ist einschichtig hochprismatisch und besteht aus zilientragenden Zellen und Hauptzellen. Aufgrund ihrer zytologischen Eigenschaften können die Hauptzellen in drei Typen unterteilt werden. Der Ductus epididymidis wird in seinem ganzen Verlauf von einem mehrreihigen hochprismatischen Epithel ausgekleidet. Das Epithel baut sich aus den schlanken, Stereozilien tragenden Hauptzellen und den kleinen Basalzellen auf. Aufgrund von einigen morphometrischen Parametern wie Epithelhöhe, Durchmesser des Kanallumens und Breite des peritubulären Muskelmantels konnte der Nebenhoden in sechs Segmente unterteilt werden.

Die ultrastrukturellen Untersuchungen zeigten einen gut entwickelten Golgi-Apparat und zahlreiche Profile des (spärlich) granulierten endoplasmatischen Retikulums im Zytoplasma der Hauptzellen. In den proximalen Segmenten tragen die apikale Oberflächen der Hauptzellen lange (17 µm) Stereozilien. Ihre apikalen Zellbreiche weisen einen gut entwickelten endozytotischen Apparat auf. Die schmalen basalen Ausläufer der Hauptzellen enthalten zahlreichen verschiedenartige Mitochondrien, Lysosomen sowie elektrondichte Granula und „residual bodies“.

Basalzellen befinden sich zwischen den schmalen basalen Ausläufern der Hauptzellen und der Basallamina. Sie haben einen nierenförmigen meistens tief eingebuchteten Kern und sind durch nur geringe Mengen an Organellen gekennzeichnet.

Apikal Mitochondrien-reiche Zellen (AMRC) wurden häufig in den Segmenten II und III und selten in den Segmenten IV und V gefunden. Ihr Hyaloplasma ist heller, als das der benachbarten Hauptzellen und ihre apikale Oberfläche ist mit kurzen Mikrovilli versehen. Abgesehen von einer mäßigen Anzahl an Mitochondrien, einem kleinen Golgi-Apparat und vereinzelten Stränge von rER, enthalten sie nur wenige Organellen.

Intraepitheliale Makrophagen werden gelegentlich im basalen Drittel des Epithels beobachtet. Sie besitzen viele Mitochondrien, einen gut entwickelten Golgi-Apparat, rER sowie kleine heterochromatinreiche Kerne. Im Zytoplasma sind viele Lysosomen und elektrondichten Restkörpern deutlich erkennbar. Intraepitheliale Lymphozyten sind durch heterochromatinreiche runde und meist eingekerbte Kerne und durch ein deutlich helleres schmales Zytoplasma gekennzeichnet. Sie werden häufig in der Nähe der subepithelialen Kapillaren angetroffen.

Fluorszeinisothiocyanate (FITC)-konjugierte Lektine (GSA-I, PNA, ECA, WGA, Con A, LCA, PSA, DBA, HPA, SBA, VVA, LTA und UEA-I) wurden für die Untersuchung der regionalen Verteilung der Saccharidgruppen im Ductus epididymidis des Rindes verwendet. Die Stereozilien der Hauptzellen in den unterschiedlichen Segmenten reagieren deutlich mit WGA, Con A, LCA und PSA. DBA- und HPA-Bindungsstellen sind nur auf die Stereozilien in der Caput Region begrenzt. WGA, LCA, PSA, DBA und HPA besitzen Bindungsstellen in der Golgi-Zone der Hauptzellen, besonders im Caput epididymidis. Die Basalzellen reagierten deutlich mit WGA, Con A, LCA, PSA und HPA. Intraepitheliale Leukozyten zeigen eine mäßige Zahl von Bindungsstellen für PNA, WGA, LCA und PSA. Die Basalmembran reagierte nur mit WGA. Das Bindegewebe im Nebenhoden zeigte eine schwache bis mäßige Reaktion mit ECA und WGA. GSA-I reagiert deutlich mit dem Gefäßendothel und kann so als Marker für das Endothel des Rindes verwendet werden. Samenzellen binden WGA und PNA deutlich. Keine Bindungsstellen konnten für VVA, LTA oder UEA-I gefunden werden.

Immunohistochemische Untersuchungen mit der ABC-Methode wurde zur Lokalisation von S-100, FGF-1, FGF-2, ACE, GT, VEGF, α-SMA, Laminin, Connexin 43, CD4, CD8 und von CD68 im Nebenhoden eingesetzt. Das Epithel der Ductuli efferentes zeigt eine intensive Immunreaktion für S-100, FGF-1 und FGF-2 und eine mäßige Immunfärbung für ACE und GT. Die Hauptzellen der ersten drei Segmente des Ductus epididymidis reagierten deutlich mit S-100. Auch FGF-1 zeigte eine deutliche Reaktion im Epithel des gesamten Ductus epididymidis. Die Hauptzellen in den ersten, zweiten und sechsten Segmenten zeigten eine intensive Immunfärbung für ACE. Die GT reagierte in der Golgi-Zone der Hauptzellen stark (Segmente II und III), deutlich (Segmente IV und V) oder schwach positive (Segmente I und VI). Basalzellen zeigten eine mäßige (FGF-1) oder intensive (FGF-2) Reaktion in den unterschiedlichen epididymalen Segmenten. Eine intensive Immunfärbung ließ sich im Endothel der Blutgefäße (ACE), in der Basallamina des Endothels (Laminin) und in den glatten Muskelzellen der Blutgefäß (α-SMA) nachweisen. Die Basallamina des Epithels und

der peritubulär glatten Muskelzellen ergab eine schwache bis mäßige Immunreaktion für Laminin. Die peritubulären glatten Muskelzellen reagierten beim immunhistochemischen Nachweis für α-SMA stark. $CD4^+$ T Lymphozyten und $CD68^+$ Makrophagen ließen sich innerhalb des Epithels des Ductus epididymidis und im Interstitium des Nebenhodens nachweisen. Mastzellen färben sich stark mit Alcian Blau und Toluidin Blau. Sie zeigten auch eine deutliche Immunfärbung für VEGF und FGF-2.

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9 ABBREVIATIONS

ABP = Androgen binding protein.

ACE = Angiotensin converting enzyme.

AEG = Acid epididymal glycoprotein.

ALPase = Alkaline phosphatase.

AMRC = Apical mitochondria-rich cells.

AR = Antigen retrieval.

BC = Basal cells.

BL = Basal lamina.

BM = Basal membrane.

cAMP = Cyclic Adenosine monophosphate.

CC = Ciliated cells of efferent ductules.

Con A = Concanavalin Agglutinin.

CRL = Crown Rump Length.

CT = Connective tissue.

Da = Dalton.

DBA = *Dolichos biflorus* Agglutinin.

DHT = dihydrotestosterone.

ECA = *Erythrina cristagalli* Agglutinin.

ECM = Extracellular matrix.

ED = Efferent ductules.

ER = Endoplasmic reticulum.

ETRT = Extratesticular rete testis.

FGF-1 = Acidic Fibroblast Growth Factor (aFGF).

FGF-2 = Basic Fibroblast Growth Factor (bFGF).

FGFRs = Fibroblast Growth Factor Receptors.

FGFs = Fibroblast Growth Factors.

FITC = Fluoroisothiocyanate.

FMP = Forward motility protein.

gACE = germinal Angiotensin Converting Enzyme.

GalNAc = N-acetyl-galactosamine.

GGT = Gamma glutamyl transpeptidase.

GJ = Gap junctions.

GlcNAc = N-acetyl-glucosamine.

GMC = Giant mesonephric corpuscle.

GPC = Glycerylphosphorylcholine.

GSA I = *Griffonia simplicifolia* Agglutinin I.

GT = Galactosyltransferase.

GZ = Golgi zone.

H&E = Haematoxylin and Eosin.

HPA = *Helix pomatia* Agglutinin.

IEL = intraepithelial lymphocytes.

IEM = intraepithelial macrophages.

LCA = *Lens culinaris* Agglutinin.

LTA = *Lotus tetragonolobus* Agglutinin.

MC = Mast cells.

MHC = Major histocompatability complex.

MVBs = Multivesicular bodies.

NC = Nonciliated cells of efferent ductules.

nm = Nanometre.

PAS = Periodic acid Schiff.

PC = Principal cells.

pcd = Post coital day.

PGC = Primordial germ cell.

PMC = Peritubular muscle coat.

PNA = Peanut Agglutinin.

PSA = *Pisum sativum* Agglutinin.

rER = rough Endoplasmic Reticulum.

RTF = Rete testis fluid.

sACE = somatic Angiotensin Converting Enzyme.

SBA = Soybean (*Glycine maximus)* Agglutinin.

SCF = Stem cell factor.

sER = smooth Endoplasmic Reticulum.

SMA = Smooth Muscle Actin.

SMC = Smooth muscle cells.

SSL = Scheitel-Steiß Länge (German synonym of CRL).

Tag p. c. = Tag post coitum (German synonym of pcd).

UEA I = *Ulex Europaeus* Agglutinin.

VEGF = Vascular Endothelial Growth Factor.

VEGFRs = Vascular Endothelial Growth Factor Receptors.

VPF = Vascular Permeability Factor

VVA = *Vicia villosa* Agglutinin.

WGA = Wheat germ Agglutinin.

º C = Degree centigrade.

µm = Micrometer.

10 CURRICULUM VITAE

I. Personal Data:

Name:	Mohamed El-Sayed El-Sayed Alkafafy
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