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# The Epididymis: Embryology, Structure, Function and Its Role in Fertilization and Infertility

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## 1. Introduction

The epididymis, located between the efferent ducts and the vas deferens, is a male accessory organ characterized by a single coiled tubule duct with an estimated length of 5-7 m in men (Sullivan, 2004; O'Hara et al., 2011).

The anatomic segments of the epididymis include the initial segment, the caput, the corpus and the cauda. Each region consists of a lumen and a polarized epithelium composed mostly of principal and basal cells (Lasserre et al., 2001; Dacheux et al., 2005). Although these four anatomical regions of the epididymis are easily identified in most adult male mammals (Yanagimachi et al., 1985; Smithwick & Young, 2001), histological and ultrastructural segmentation of this organ varies among the different phylogenies of mammals. The rat epididymis is most commonly adopted as an experimental model of study (Figure 1).

Several descriptive anatomical and histological studies of the epididymis appeared at the beginning of the twentieth century. The authors hypothesized that the epididymal secretions played a role in the maintenance of sperm vitality, sperm motility (Benoit, 1926) and the capacity to become fertile (Young, 1929a, 1929b, 1931). Relatively little research was done on the excurrent duct system during the ensuing three decades. However, in 1967, Marie-Claire Orgebin-Crist demonstrated that the key event in sperm maturation was not the passage of time but the exposure of the sperm to the luminal environment of the epididymis (Bedford, 1967; Orgebin-Crist, 1967).

The epididymal duct is now recognized as a channel that transports, concentrates and stores the spermatozoa. It is also known that the spermatozoa leaving the testis are immovable, immature and unable to fertilize an oocyte (Yanagimachi, 1994; Flesch & Gadella, 2000), and that under androgen control, the epididymal epithelium secretes proteins within the intraluminal compartment that create a very complex environment surrounding the spermatozoa (Hermo et al., 1994, 2004; Sullivan, 2004). This luminal compartment stores the spermatozoa until ejaculation and specifically prepares the sperm for fertilization by providing the essentials in terms of temperature, oxygen tension, pH and an available energy substrate (Dacheux et al., 2005). The epididymal duct produces the morphological,

biochemical, physiological and functional changes to the structures of the spermatozoa through a process known as epididymal maturation, which converts the spermatozoa into fertilization-competent cells (Toshimori, 2003; Gatti et al., 2004; Flesch & Gadella, 2000).

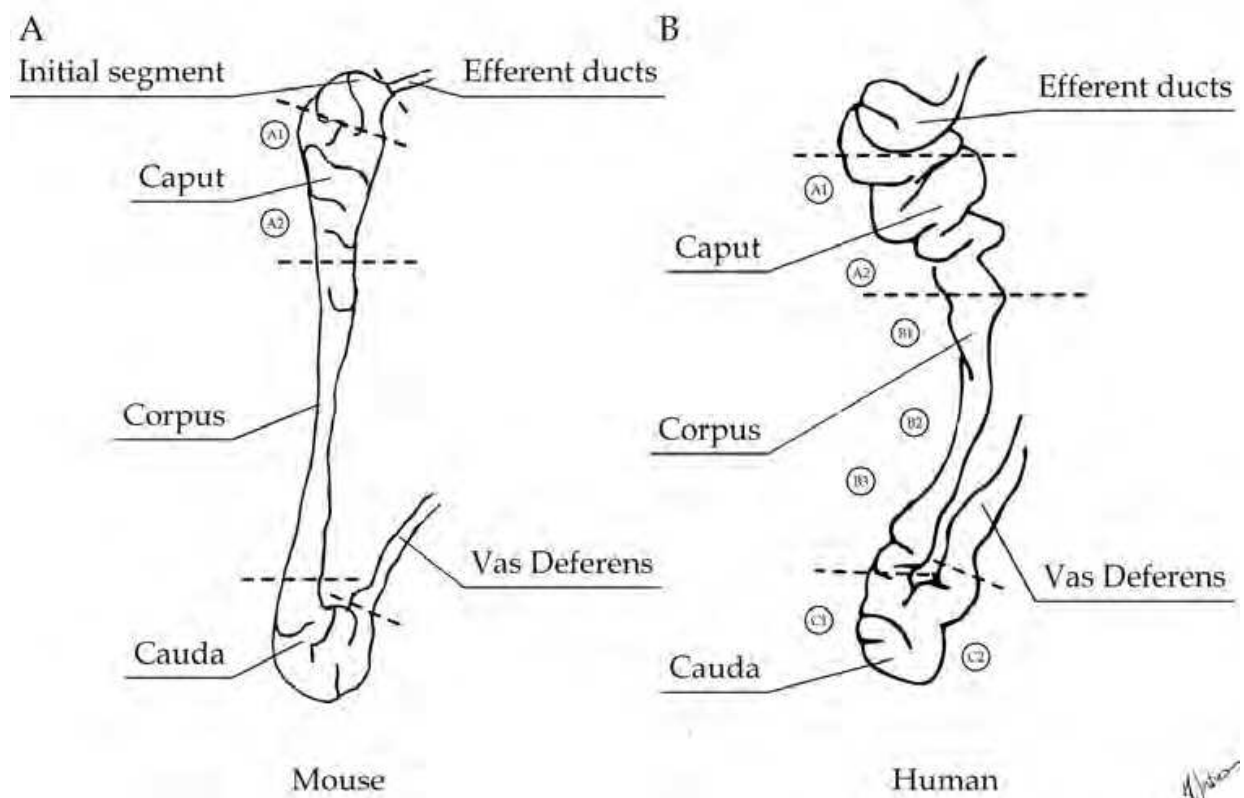


Fig. 1. The regionalization of the epididymis. The illustrations compare the mouse and human epididymides. A) Mouse epididymis (A1: proximal caput, A2: mid-caput). B) Human epididymis (A1: anterior caput, A2: posterior caput, B1: anterior corpus, B2: mid-corpus, B3: posterior corpus, C1: anterior cauda, C2: posterior cauda).

Since 1966, more than 12,000 research articles have been published on the epididymis. These articles, while addressing the various aspects of the epididymis from several points of view, all agree that the epididymis is crucial for the preparation of the spermatozoa prior to fertilization. It is well known that the proteins and small molecules secreted by the epididymal epithelium into the lumen interact with the transiting spermatozoa and directly or indirectly affect the spermatozoa surface; these proteins and small molecules function as signaling molecules to induce activity in the other epididymal proteins (Gatti et al., 2004). A significant number of these molecules will be taken up by the spermatozoa. Several of the acrosome molecules, which were previously formed during spermiogenesis and are involved in the acrosome reaction or in the sperm-zona pellucida interaction and sperm-egg fusion, will be gradually rearranged and compartmentalized in a stage-specific manner during sperm maturation (França et al., 2005).

Through research, we have learned a substantial amount about the function and the biochemical properties of the epididymal structure. However, there are still gaps in nearly all aspects of the research. Studying the complexity of the cellular properties, the spatial and temporal organization of protein syntheses and secretion and the dynamic interactions

between the epithelial cells and the contents of the luminal compartment remains challenging but important. A more complete understanding of this organ will allow for the development of male contraceptive agents (Turner et al., 2006). Additionally, up to 40% of infertile men exhibit idiopathic infertility that may reflect sperm maturational disorders (Cornwall, 2009).

A greater understanding of the function and biochemical properties of the epididymis may lead to the development of therapeutic agents to treat certain types of infertility. This chapter provides an overview of the human epididymal embryology, malformation, structure and function.

## 2. The prenatal development of the epididymis

In mammals, the genetic sex of the embryo is determined mainly by the presence or absence of a single gene on the Y chromosome, the SRY, which is required to initiate male-specific pathways and to repress female-specific pathways of development (Moore & Persaud, 2003). At the onset of sex differentiation, the reproductive primordial organs are indistinguishable in male and female embryos. The gonads (testicles or ovaries), the genital ducts (Wolffian or Müllerian) and the urogenital sinus of both sexes emerge from the morphologically undifferentiated primordia. We can then assume that the genes involved in the establishment of these primordia are the same for both sexes. During development, the first morphological sex difference appear in the gonads of male embryos, followed by the genital ducts, and finally, they appear in the urogenital sinus (Larios & Mendoza, 2001).

In humans, the genital ducts arise from the intermediate mesoderm. Shortly after the formation of the Wolffian ducts, the adjacent Müllerian ducts are formed. In males, sexual differentiation develops through regression of the Müllerian ducts and the development of the Wolffian ducts into multiple reproductive organs, such as the epididymis, the vas deferens ducts and the seminal vesicles. In females, the Wolffian ducts regress and the Müllerian ducts differentiate to form the uterus, the fallopian tubes, and the proximal vagina (Hannema & Hughes, 2007; Kobayashi & Behringer, 2003).

The development of sexual differentiation involves genetic processes that are primarily controlled by the sexual chromosomes (França et al., 2005).

In the male tract, Müllerian duct regression, which depends on the testicular Sertoli cells, is mediated by the Y chromosome genes and is triggered by a mechanism that is not well understood. The Sertoli cells produce and secrete an anti-Müllerian substance, a non-steroidal hormone. Under the control of human chorionic gonadotrophin (hCG), a placental gonadotrophin, the Leydig cells then differentiate and produce the androgens that will positively regulate the ipsilateral Wolffian duct in a paracrine way. These hormonal combinations induce the cranial Wolffian duct to become highly convoluted and to differentiate into the epididymis.

The major morphogenic event during Wolffian duct/epididymal duct development is the elongation and coiling of the duct (Hannema & Hughes, 2007). The process of elongation is likely a product of potential mechanisms such as cell proliferation coupled with directed cell rearrangements, along with the interactions between the Wolffian duct epithelium and the surrounding mesenchyme cells (Hinton et al., 2011).

The Wolffian ducts also induce the formation of the mesonephric tubules in the mesonephric mesenchyme, which extend to the epithelial cells of the gonad and subsequently differentiate into efferent ductules. These ductules open into the epididymis (Figure 2). Distal to the epididymis, the Wolffian ducts acquire a thicker investment of smooth muscle and become the ductus deferens (Moore & Persaud, 2003; Hannema & Hughes, 2007).

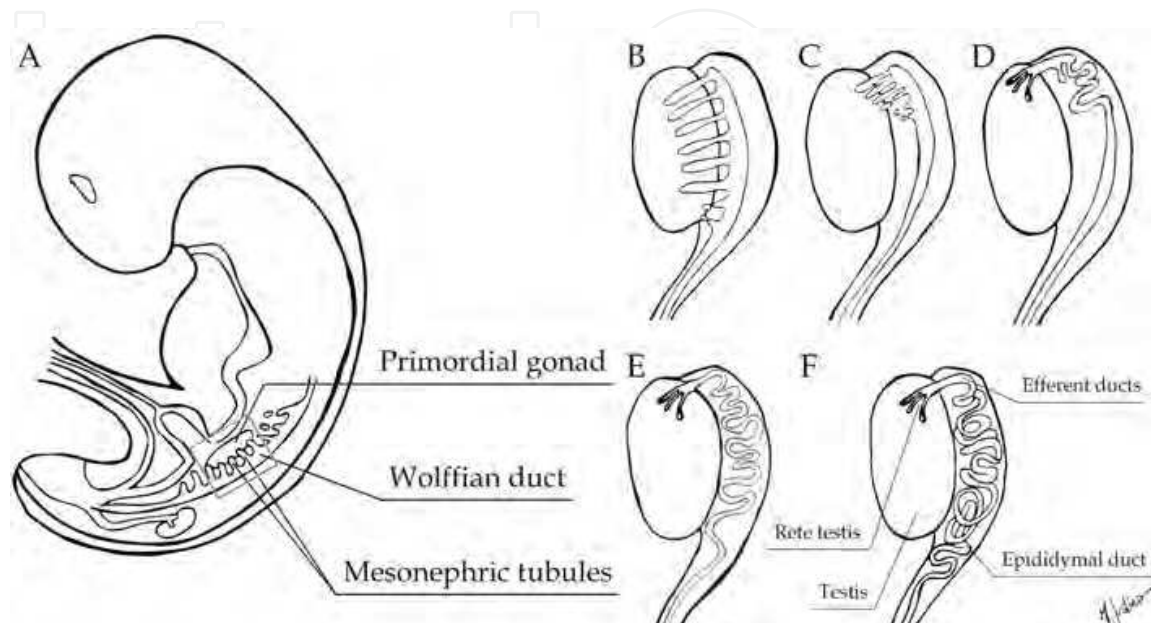


Fig. 2. The intra-uterine development of the epididymis. A) A sagittal section of a mouse embryo showing the relative locations of the developing urogenital structures. B) The testicular production and secretion of testosterone positively regulates the Wolffian ducts. The ducts then induce the formation of the mesonephric tubules in the mesonephric mesenchyme that extend to the epithelial cells of the gonad. C) The cords arising from the apical mesonephric tubules form the efferent ducts and fuse with the adjacent ducts to form the rete testis. The coiling of the initial segment of the efferent duct is not shown here, but it proceeds independently of the coiling of the main epididymal duct shown in D, E and F. D) The coiling shifts from the proximal to the distal duct in a temporal fashion. E) The initial stages of coiling are planar, meaning that the coiling is at the two-dimensional level. F) The three-dimensional coiling proceeds in a caput-to-cauda direction and is completed in the early postnatal period.

### 3. The postnatal development of the epididymis

While cell proliferation in the Wolffian duct appears to be dependent on the presence of androgens and mesenchymal factors during prenatal development, lumicrine factors produced by the testicles play an additional role during postnatal development (Hinton et al., 2011). Lumicrine factors are molecules (such as androgens, growth factors and enzymes) whose effect on the secretory activity of the epithelial cells of the epididymis and in the epididymis spermatozoa directly participates in the process of epididymal maturation (Lan et al., 1998; Robaire et al., 2006). In general, the postnatal development of the mammalian epididymis can be divided into three periods that take place across two developmental phases (Figure 3).



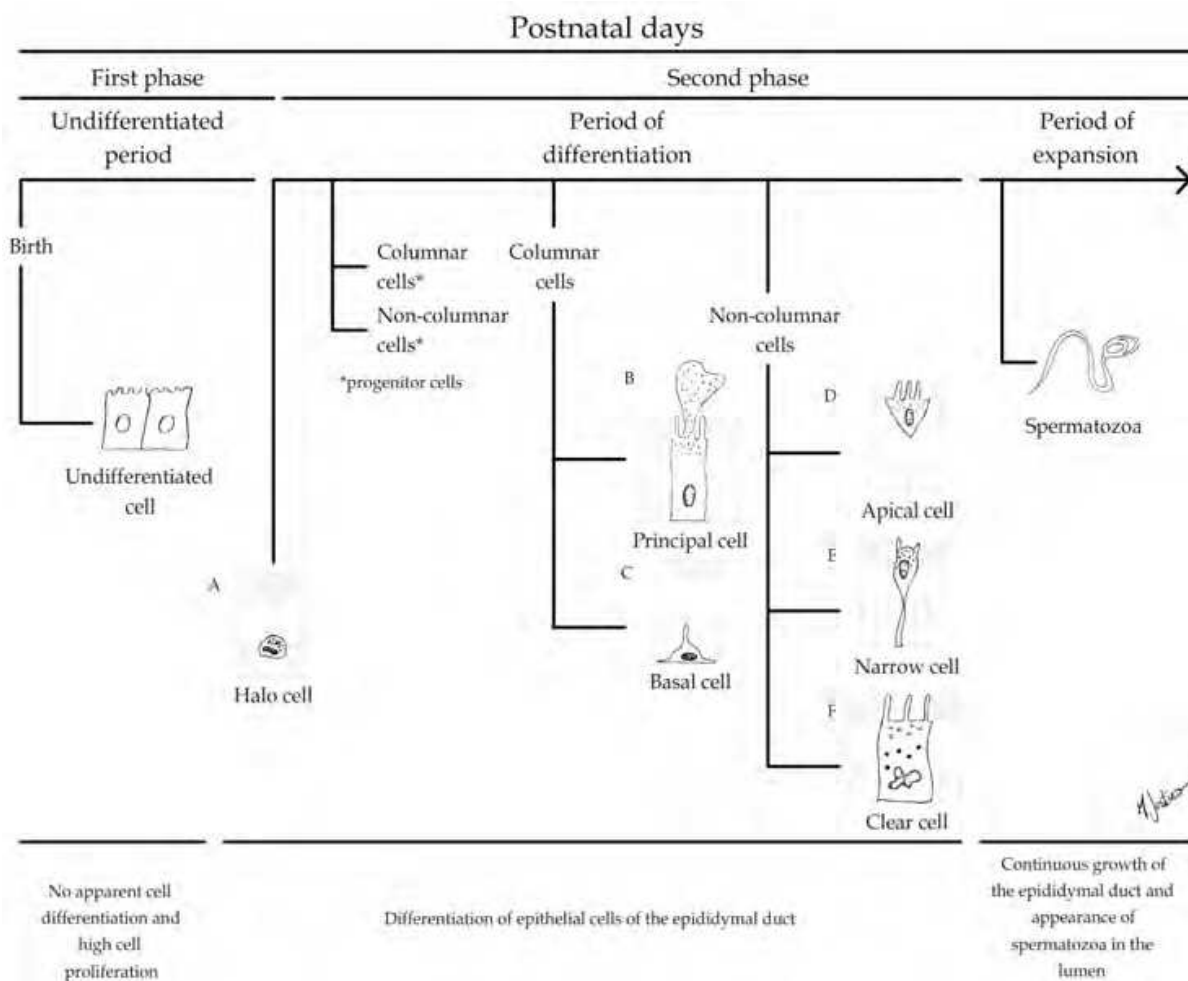


Fig. 3. A schematic diagram that describes the differentiation of the mouse epididymal epithelium from birth until adulthood. A to F indicate the different types of cells in the epithelium of the epididymis.

### 3.1 The first phase: From birth to early infancy

By birth, the presumptive areas of the initial segment, caput and corpus have experienced considerable coiling compared with the epididymal cauda (Figure 4). Within the first few days after birth, the individual conjunctive septum initiates the anatomical segmentation of epididymis, along with the considerable growth of the duct. The epithelium is still undifferentiated and characterized by columnar cells. The histological changes do not begin until the first stages of early infancy with the appearance of halo cells (Robaire et al., 2000, 2006) (Figure 3). The first phase begins, then, with the undifferentiated period and ends with the first event of the differentiation period, which is defined by the appearance of halo cells.

### 3.2 The second phase: From early infancy to adulthood

The epididymal epithelium differentiates into the pseudostratified epithelium, which contains principal, basal, apical, narrow and clear cells (Figure 3). Epithelial cell differentiation is completed during puberty when the highest rate of cell division and epididymal expansion occurs. This period of expansion describes the continued growth of

the duct and the appearance of spermatozoa in the epididymal lumen. The establishment of the regionalized protein secretion takes place progressively coupled to the consolidation of an anatomical epididymal segmentation (Figure 4). The differentiation and regionalization are related to the different stages of testicular maturation, the steroidogenic activity of the Leydig cells, the androgen dependence of the epididymis itself and the lumicrine factors (De Miguel et al., 1998; Robaire et al., 2000; Dacheux et al., 2005; Cornwall, 2009; Robaire et al., 2006). Then the second phase starts after halo cell differentiation and ends in puberty.

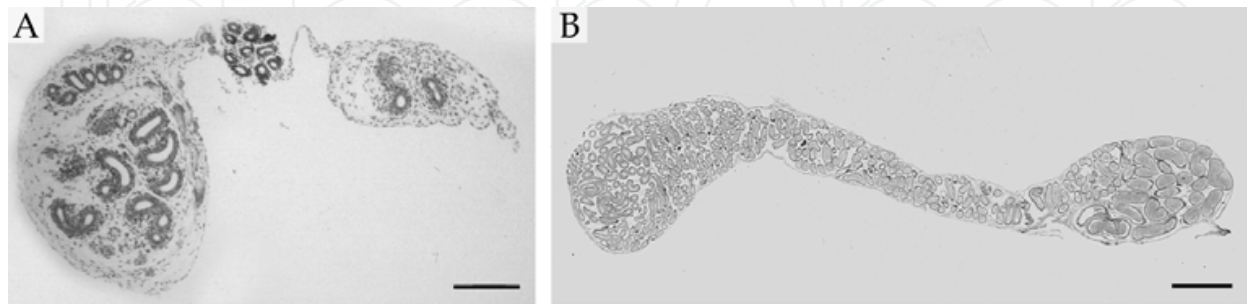


Fig. 4. Photomicrographs of a mouse epididymis. A) At birth, the undifferentiated period shows a poorly coiling duct and little regionalization. B) A well-formed adult epididymis in expansion with a completely coiled and regionalized duct. Scale bar: A) 100 $\mu$ m. B) 1100 $\mu$ m.

#### 4. Structural features

In the testes, the seminiferous tubules converge to form the rete testis, which in turn gives rise to the efferent ducts. The number of tubules contained in the efferent ducts varies depending on the species and on how the tubules converge to form the single coiled duct of the epididymis. The epididymal tubular lumen is continuous with the lumina of the efferent ducts and comes to an end in the vas deferens, which has a thick layer of muscle. The urethra is the final extension of the vas deferens and communicates with the outside of the body.

##### 4.1 Macroscopic features

The mammalian epididymis is an elongated coiled duct suspended within the mesorchium and firmly or loosely bound to the testicular tunica albuginea. The gross aspect of the epididymis allows the identification of the different segments (Figure 1), which are comprised of the proximal region (the initial segment and the caput), the corpus and the distal cauda (Robaire et al., 2006; Turner, 2008). In all mammalian species, each region of the epididymis is further organized into lobules that are separated by the connective tissue septa. These septa not only serve as internal support for the organ but are also thought to provide a functional separation between the lobules that allows for the selective expression of genes and proteins within the individual lobules (Kirchhoff, 1999; Cornwall, 2009).

##### 4.2 Microscopic features

The epididymis also contains region-specific and cell-specific functions specifically located within the epithelium of a given segment. The highly specific regionalization of the

epithelium and the luminal protein secretion within the three main epididymal segments can be further subdivided into several regions (França et al., 2005; Robaire et al., 2006; Turner, 2008). In general, the five physiological regions are distinguished as follows: (i) the proximal caput or the initial segment, (ii) the middle caput, (iii) the distal caput and the proximal corpus, (iv) the distal corpus and (v) the cauda (Dacheux et al., 2005; Turner, 2008). Note that the proposed physiological regions are slightly different from the anatomical regions (Figure 1).

The differential response of the segments to androgen withdrawal, stress and aging indicates that each region represents discrete regulatory units (Jervis & Robaire, 2001). The epididymis is more than a uniform channel that transports and stores spermatozoa. The maturation and storage of the spermatozoa depends upon the epithelial tight and adhering junctions. They are important in maintaining the integrity of the epididymal epithelium and in the formation of the blood epididymal barrier. Tight junctions between the adjacent epididymal epithelial cells form the blood-epididymal barrier and restrict the passage of a number of ions, solutes, and macromolecules through the epididymal epithelium. This barrier also serves as an extension of the blood-testis barrier. The spermatozoa are immunogenic and contain proteins on their surfaces that would be recognized as nonself if they leave the epididymis (Robaire & Hermo, 1988; Dacheux et al., 2005).

Histological characteristics allow for the easy identification of the anterior and posterior extremities of the mammalian epididymis. The thickness of the epididymal epithelium varies with the thickest portion in the proximal caput and the thinnest in the caudal region (Figure 5). Conversely, the luminal diameter and the thickness of the peritubular smooth muscle increases from the proximal to the distal regions (Lasserre et al., 2001; Toshimori, 2003). Few sperm are found in the initial segment, but a large mass of sperm aggregates are located in the cauda (Yanagimachi et al., 1985; Cornwall, 2009). In all of these segments, the epididymal duct is lined with an epithelium composed of principal and basal cells. Other cells, such as apical, narrow, clear and halo cells, are also present in this duct in a segment-specific manner (Figure 6).



Fig. 5. Photomicrographs showing the histological regionalization of the A) initial segment and caput separated by the connective tissue septa, B) the corpus and C) the cauda of the mouse epididymis. The thickness of the epididymal epithelium varies from the thickest portion in the proximal caput to the thinnest in the caudal region. Conversely, the luminal diameter and the thickness of the peritubular smooth muscle increases from the proximal to the distal regions. Scale bar: 50 $\mu$ m.



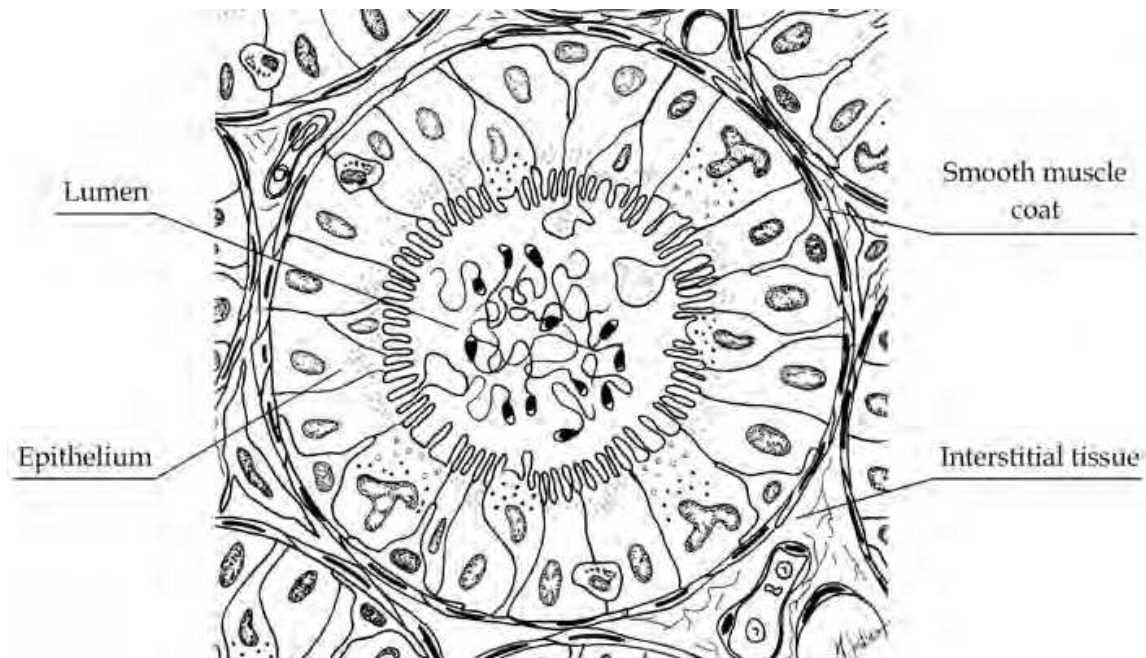


Fig. 6. A schematic cross section of epididymis showing the organization of the major cell types in the epithelium of the epididymal duct as observed through a light microscope. A generic epididymal compartment with the relative position and distribution of all cell types found throughout the epithelium is illustrated. The thinnest coat of organized smooth muscle is in the interstitial tissue surrounding the duct and is characteristic of the anterior regions of the epididymis. Note the spermatozoa in the center of the epididymal lumen.

#### 4.2.1 Principal cells

Principal cells (Figure 3B) are the most abundant and extensively studied cell type found in the epididymal epithelium. Principal cells constitute approximately 80% of the total epithelial cell population in the initial segment. The number of principal cells gradually decreases to 65% of the total epithelial cell population in the cauda epididymis (Robaire & Hermo, 1988). These columnar cells present prominent stereocilia and extend into the lumen. Ultrastructurally, the supranuclear region of this cell type contains large stacks of Golgi saccules, mitochondria, multivesicular bodies and apical dilated membranous elements, while the infranuclear region is densely packed with rough endoplasmic reticulum (Robaire et al., 2000, Dacheux et al., 2005). Principal cells are responsible for the bulk of the proteins that are secreted into the lumen and are directly involved in the control of luminal protein concentrations. They frequently exhibit blebs of cytoplasm emanating from their apical cell surface. These cells also form tight junctions with one another, and as such, form the blood-epididymis barrier (Robaire et al., 2006; Cornwall, 2009).

#### 4.2.2 Basal cells

Basal cells (Figure 3C) are the second most abundant cell type found in the epididymal epithelium, constituting 15-20% of the total epithelial cell population of the epididymis. They are triangular and flat cells and they reside in the base of the epithelium. Basal cells cannot access the luminal compartment. They have elongated or round shaped nuclei, and

they are in close association with the overlying principal cells or other basal cells through the presence of cytoplasmic extensions (Robaire et al., 2000; Cornwall, 2009). Because of this contact with the basement membrane, basal cells form an extensive cellular sheet surrounding the epididymal epithelium (Robaire et al., 2006; Cornwall, 2009). Although basal cells are extratubular in origin, some findings have suggested that the cells may have a role within the processes of the epithelial immune system and in the regulation of electrolytes by principal cells. However, the exact functions of these cells are not yet clear (Robaire et al., 2006).

#### 4.2.3 Apical cells

Apical cells (Figure 3D) comprise approximately 10% of the total epithelial population in the initial segment but only approximately 1% of the total epithelial population in the cauda of the epididymis (Adamali & Hermo, 1996). They are clearly defined by the many mitochondria in the apical cytoplasm, the few microvilli at the luminal border and a nucleus that is located in the upper half of the cell cytoplasm (Adamali & Hermo, 1996; Robaire et al., 2006). These cells are related to sperm quiescence and to the regulation of the pH in the lumen through the production of enzymes of the carbonic anhydrase family (Hermo et al., 2005).

#### 4.2.4 Narrow cells

Narrow cells (Figure 3E) are the slender elongated cells. They increase from 3% of the total epithelial population in the initial segment to 6% of the total epithelial population in the corpus. These cells presents numerous C-shaped vesicles and mitochondria with a small flattened nucleus located in the upper half of the cell cytoplasm. The structural features of both apical and narrow cells suggest that these cells are involved in the process of intracellular transport between the lumen and the epithelial cells, in the degradation of specific proteins and carbohydrates within their lysosomes and in protecting spermatozoa from a changing environment of harmful electrophiles (Adamali & Hermo, 1996; Robaire et al., 2006). They also differ dramatically from the neighboring principal cells and display region-specific expression of proteins, such as the glutathione S-transferases and lysosomal enzymes (Adamali & Hermo, 1996).

#### 4.2.5 Clear cells

Clear cells (Figure 3F), along with halo cells, constitute fewer than 5% of the total epithelial cell population. Clear cells are equally distributed through the caput, the corpus and the cauda segments. The dark-stained nucleus of these cells is surrounded by the pale-staining cytoplasm. They are also present in all levels of the epididymal epithelium. Clear cells are also endocytic cells and may be responsible for the clearance of proteins from the epididymal lumen. They normally take up the contents of the cytoplasmic droplets released by the spermatozoa as they transit through the duct (Hermo et al., 1994, 2005; Robaire et al., 2006).

#### 4.2.6 Halo cells

Halo cells (Figure 3A) are usually located in the base of the epithelium where it does not touch the basement membrane. These cells contain variable numbers of dense core granules.

They develop in the immune system from a combination of B and T lymphocytes and monocytes (Dacheux et al., 2005; Robaire et al., 2006).

#### **4.2.7 Cell interactions in the epididymal duct**

The cell types described above are active in the processes of epididymal function, such as protein secretion and absorption (principal cells); endocytosis (clear and apical cells); the secretory activities responsible for the acidification of the luminal fluid (clear cells and narrow cells); immune defense; phagocytosis (halo cells); and the production of antioxidants (basal cells) (Robaire et al., 2000; França et al., 2005). It is important to understand that each cell type may express different proteins within the distinct epididymal regions. This indicates that the cells perform different functions according to their location, and that the specificity of epididymal secretions is progressively established with age (Robaire et al., 2000, 2006). This information confirms the high degree of regionalization involved in the activity of the epididymis. This epididymal regionalization, which is attributed to the diverse patterns of gene expression, is critical to the formation and maintenance of the functions of the epididymal duct (Suzuki et al., 2004).

### **5. The maintenance of the epididymis**

In mammals, the development and maintenance of a fully differentiated epididymal epithelium is dependent on a combination of factors that provide an ideal site where the spermatozoa undergo a series of morphological, biochemical and physiological changes. During this process of epididymal maturation, the spermatozoa move along the ducts in a fluid that dynamically evolves through the processes of absorption and secretion by the epithelial cells, androgens, as well as through lumicrine factors from the testis (Cornwall, 2009).

#### **5.1 Hormones**

Androgens play a crucial role in the development of the male reproductive organs, such as the testis, the epididymis, the vas deferens, the seminal vesicle, the prostate and the penis. The role of androgens is an important topic in the study of puberty, male fertility and male sexual function. The effects of androgen withdrawal have been well established through the experimental model of orchiectomy. A decrease in the weight of the epididymis has been commonly observed in animals that have had their testicles removed. In these cases, androgen replacement, even at supraphysiological levels, only partially restored the weight of the epididymis. The removal of the testicles caused the loss of androgens, but it is clear that this approach affected estrogen levels and other testicular factors that may affect the maintenance of epididymis (Robaire et al., 2000).

##### **5.1.1 Androgen: Testosterone and dihydrotestosterone**

The formation and function of the epididymis is androgen-dependent. The principal androgen, testosterone (T), is essential for the development of the internal sex organs and is derived from the Wolffian duct system, which consists of the epididymis, the vas deferens, and the seminal vesicle (Umar et al., 2003). Dihydrotestosterone (DHT), the 5 $\alpha$ -reduced form

of T, is involved in the development of the prostate and the external genitalia. Although T is the predominantly active androgen during the first phase of the postnatal development of the epididymis, it is the effects of DHT that are important in the epididymal fluid of the mature epididymis. DHT can be produced locally in the epididymis by principal cells and is primarily found in the initial segment of the duct (Dacheux et al., 2005; França et al., 2005; Robaire & Henderson, 2006).

The actions of both T and DHT are initiated through the intracellular receptor known as the androgen receptor (AR). DHT is the more potent androgen of the two. The AR is found in all male reproductive organs and can be stimulated by either T or its more potent metabolite, DHT. The binding of either T or DHT to the AR may regulate distinct androgenic effects in target tissues. Clinical syndromes, such as androgen insensitivity (AIS), illustrate the differential actions of T and DHT (Umar et al., 2003). AR expression in the developing male genital tract occurs in a strict temporal pattern. It is first detected in the mesenchymal cells, then in the epithelial cells and then in both the epithelial and stromal compartments of the epididymis (Umar et al., 2003; O'Hara et al., 2011).

The initiation of androgen-dependent differentiation of the Wolffian duct system into epididymis occurs before epithelial cells express a detectable level of the AR protein. In this phase of development, the mesenchymal cells are important androgen targets that elicit androgenic effects in the epithelial cells via paracrine factors and mesenchymal-epithelial interactions (Umar et al., 2003). Genetic mutations in the AR or treatment with AR antagonists during the male embryonic stage results in the regression of the Wolffian ducts and an absence of the epididymis in adult males (O'Hara et al., 2011).

During postnatal development, the luminal secretion of androgens is essential for the maintenance of epithelial cell identity (O'Hara et al., 2011), and for the normal development and function of the stromal cells (Nitta et al., 1993; Robaire et al., 2000; Hess et al., 2001; O'Hara et al., 2011). Both the regionalized differentiation of the epididymis and the variation in the luminal fluid composition take place under the control of androgens (Toshimori, 2003). The production and secretion of at least half of the epididymal proteins, including those later incorporated by the spermatozoa in transit, are under androgenic control. Androgenic control may act positively or negatively, depending on the varying levels of sensitivity (Tezon et al., 1985; Ellerman et al., 1998; Robaire et al., 2000; Dacheux et al., 2005; Robaire & Henderson, 2006).

### 5.1.2 Estrogen

In addition to testosterone and other androgenic-derived metabolites, estrogen has been reported to target epididymal epithelial cells (Hess et al., 2001, 2011). The presence of two estrogen receptors (ESR) types in the head of the epididymis, as well as in other regions of the epididymis, has been well documented. Their expression appears to be isotype-, species-, and cell-specific (Hess et al., 2001, 2011). Recent studies have shown that the luminal fluid reabsorption that occurs in the efferent ductules and in the initial segment of the epididymis is regulated by estrogen. The estrogen present in the epididymis also regulates the transport of fluid through the duct and is responsible for increasing the concentration of sperm as they enter the caput of the epididymis (França et al., 2005; Hess et al., 2011). Estrogen assists in the maintenance of a differentiated epithelial morphology,



which means that it is absolutely necessary for the process of enhancing fertility in the male (Kobayashi & Behringer, 2003).

## 5.2 Lumicrine factors

Lumicrine factors are molecules produced by an upstream set of cells through a luminal or ductal system in the testes that actively participates through paracrine signaling to the cellular mechanisms of development and maintenance of the epididymis (Lan et al., 1998). In the absence of lumicrine factors, the segments of the epididymal duct regress to a transcriptionally undifferentiated state, which is consistent with a less differentiated histology. The absence of testicular molecules could also stimulate an individual's gene expression in some epididymal segments while suppressing it in others (Turner et al., 2007). Androgen replacement in the experimental models of castrated subjects has shown that apoptotic cell death in the epididymis can be prevented, but the initial segment is dependent on the luminal components coming from the testis and not just on the androgens alone (Robaire et al., 2000). The direct influence of lumicrine factors, in addition to the secretion of androgens by the testicular Leydig cells, is of extreme importance for both cell signaling and the maintenance of the male reproductive tract epithelia. Lumicrine factors regulate the secretion of the molecule-specific androgen-region substances present in the epididymal fluid, which support a peculiar microenvironment that is necessary for the survival and functionality of sperm (Turner et al., 2007; von Horsten et al., 2007).

## 5.3 Additional molecules

Other substances for which the receptors for or the substance itself have been found in the epididymis include prolactin, retinoic acid, and vitamin D, whose active metabolite is synthesized primarily in the cauda epididymis (Robaire et al., 2000). In a complementary way, another regulator of epididymal function is vitamin E, which plays an important role in maintaining the viability and the functional and structural appearances of the epithelial cells of the epididymal duct (França et al., 2005).

## 6. Biological function in adulthood

The epididymal functions of transporting, concentrating, maturing, and storing spermatozoa are important processes for male fertility, and their absence or depletion might be a significant factor in male infertility (Turner, 2008). In order to fulfill so many functions, the epididymis is dependent on the establishment of a peculiar microenvironment, which is formed by the highly regionalized secretion of proteins, glycoproteins and other molecules from the testis. Although the composition of the epididymal luminal fluid of several species is known to be the epididymal site where spermatozoa mature and are stored, the manner in which the epididymis contributes to the formation of this specialized milieu is not fully understood (Robaire et al., 2006). Our understanding of the mechanisms behind the regulation of the functions of the epididymal epithelium, and their effects on the spermatozoa, is still fairly limited. The major function of the adult epididymis is to provide the ideal conditions that ensure the progress of the spermatozoa along the duct as they are exposed to a continually changing environment that supports the development and maintenance of fertilization.



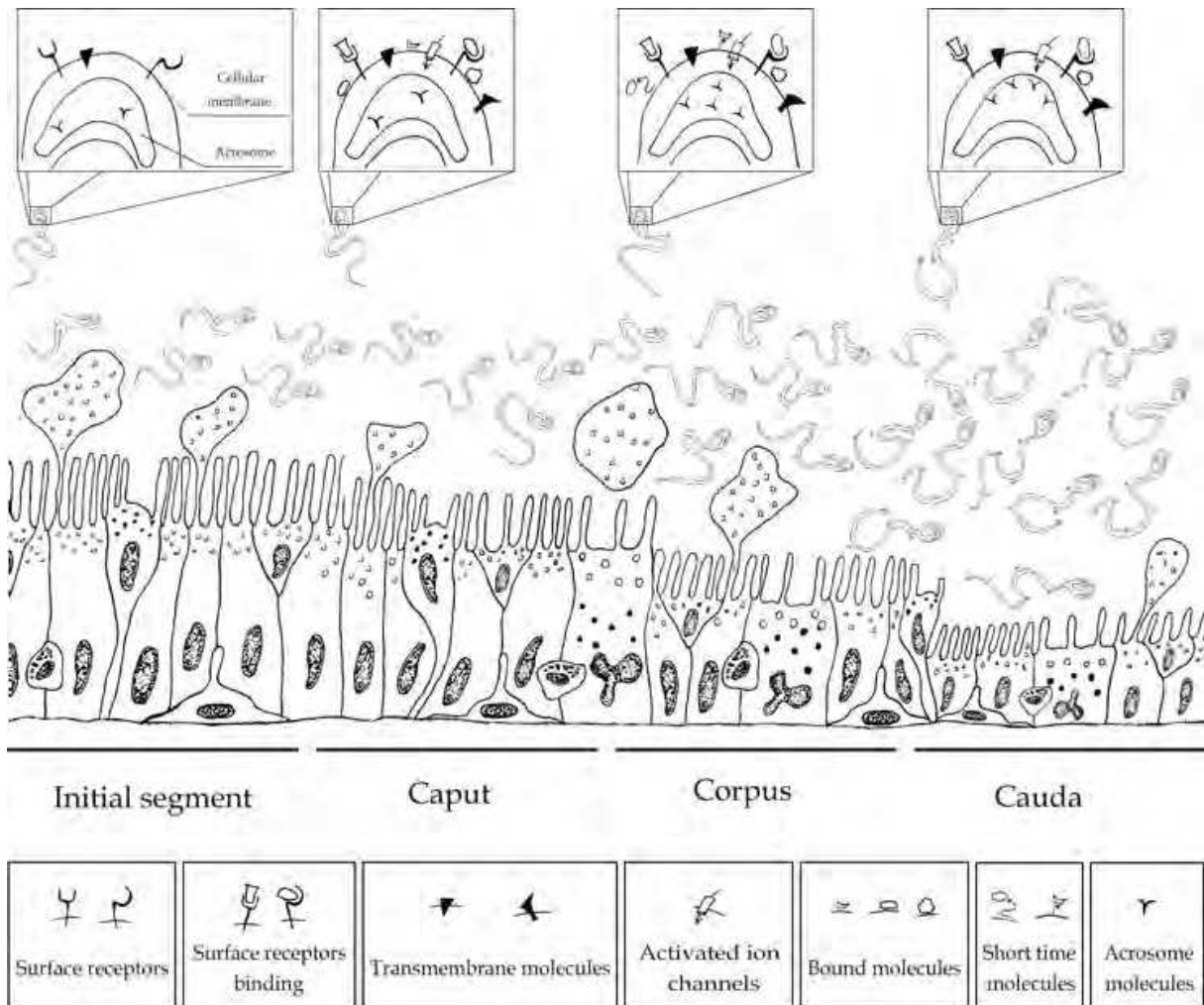


Fig. 7. A schematic illustration representing the distinct changes in the sperm surface throughout the stages of sperm maturation and epididymal cellular distribution throughout the epididymal epithelium. Histological characteristics allow for the identification of the anterior and posterior extremities of the epididymis. The epididymal epithelium varies from its thickest section in the proximal caput to its thinnest section in the caudal region. The relative position and distribution of the main cell types are illustrated in each macroscopic structural region of the epididymis demonstrating that principal and basal cells are the two most abundant cell types throughout the entire epididymis. Detailed illustrations in the upper portion of the figure show the maturation of the spermatozoa, along with the incorporation of the molecules along the passage through the duct. Some molecules are bound, and later, may or may not disappear. Other molecules are incorporated into the cellular membrane of the plasma of the spermatozoa or associate with the surface receptors and ions channels that are activated. Note the molecules that are also incorporated into the acrosomal vesicle. Some of these molecules will persist until the capacitation reaction in the reproductive female tract. These molecules are indispensable to the specific recognition processes of the egg-spermatozoa interaction.

### 6.1 The epididymal maturation of the spermatozoa

Sperm maturation involves morphological and biochemical changes in the sperm surface in response to the epididymal secretions of enzymes, proteins and glycoproteins, which are essential in the process of fertilization (Orgebin-Crist, 1967; Robaire et al., 2000, 2006). The knowledge of the interactions between the luminal fluid microenvironment and the dynamics of the epididymal epithelium is indispensable to an understanding of the process of the development and maturation of the spermatozoa in the adult epididymis.

In the process of sperm maturation, molecules are secreted into the luminal fluid by different regions of the epididymis. These molecules interact sequentially with the surface of the spermatozoa or the acrosome, and alter their molecular function (Orgebin-Crist, 1967; Robaire et al., 2000, 2006; Gatti et al., 2004; Dacheux et al., 2005; Sullivan et al., 2005) (Figure 7). In this process, some proteins bind to the sperm and presumably affect sperm function directly (Ellerman et al., 1998; Von Horsten et al., 2007). Some proteins will later bind to the zona pellucida (Ellerman et al., 1998) or the plasma membrane of the oocyte (Cohen et al., 1996; Flesch & Gadella, 2000). Other proteins remain in the lumen throughout the length of the tubule (Dacheux et al., 2005; Fouchécourt et al., 2002). Several of the epididymal proteins that remain in the lumen are either closely bound to each other or they may be integrated into the membrane of the spermatozoa because of their hydrophobic properties (as GPI-anchored proteins) or because of the proteolysis of their carboxy-terminal region (Gatti et al., 2004).

Other proteins are present in the epididymal lumen for only a short time, suggesting that their continued presence may be detrimental to sperm maturation and/or epididymal cell functions. Selective mechanisms are in place for their removal (von Horsten et al., 2007). These short-acting proteins that are loosely bound probably prevent direct interactions with the sperm surface by masking the sites that will be activated during fertilization.

In addition, there are a number of proteins that can be transferred from the epididymal epithelium to the spermatozoa by a very specific and not fully elucidated mechanism mediated by the epididymosomes. Almost all of these proteins are rapidly absorbed or degraded in the first segment of the epididymis. Some of these molecules or aggregates become outer membrane components of the sperm, while others become integral membrane proteins. CRISPs (Cysteine-Rich Secretory Proteins, originally named DE) are epididymal proteins in rats and mice that do not require stable or long-term bounds with the sperm to perform their functions. CRISPs adhere to the sperm surface as epididymosomes during epididymal maturation and are subsequently lost during epididymal transit (Ellerman et al., 1998; Cohen et al., 2011).

Some molecules secreted by the epididymis do not interact directly with the surface of the sperm. Instead, the molecules interact with the sperm acrosome. The sperm acrosome is a highly specialized organelle overlying the anterior part of the sperm nucleus. The acrosome contains a number of hydrolytic enzymes that are believed to be required for fertilization (Yoshinaga & Toshimori, 2003).

The SP-10 and the acrin family (1 and 2) are examples of molecules undergoing intense regulation of the distribution in the movement into and out of the acrosome. These molecules induce changes in the acrosomal proteins of the spermatozoa by maturation-

dependent modifications attributed to glycosylation/deglycosylation and/or proteolytic processing during epididymal maturation (Yoshinaga & Toshimori, 2003). The acrosomal human SP-10 protein decreases from 45 kDa to 18-25 kDa as a result of the proteolytic process when the spermatozoa pass into the caput epididymis. Acrin 1 and acrin 2 move from one acrosomal domain to another, which results in a compartmentalization of these molecules during epididymal maturation. Intra-acrosomal proteins initially generated in the testis can change their location and their size simultaneously during epididymal maturation (Yoshinaga & Toshimori, 2003).

There is a range of others proteins that are known to dwell in the epididymal fluid, such as lactoferrin, clusterin, cholesterol transfer protein, glutathione-peroxidase, albuminoidal proteins, prostaglandin, prostaglandin D2 synthase, hexosaminidase and procathepsin D (Fouchecort et al., 2000; Suzuki et al., 2004). It is generally known that these epididymal-secreted proteins exhibit either transport and binding functions or enzymatic activity. They contribute to the fertilization capacity of the spermatozoa by facilitating the exchange of proteins or lipids between the spermatozoa and the surrounding fluid (Dacheux et al., 2005) during epididymal maturation. The proteolysis of the pre-existing proteins, and even the metabolic activity of the spermatozoa, also contributes to the protein profile of the epididymal fluid (Dacheux et al., 2005).

Most of the molecules found in the epididymal luminal fluid participate in the maturation of the spermatozoa by responding to one of the various mechanisms previously described. Although the many of the molecular mechanisms that induce sperm maturation have not been definitively identified, it has been established that the regionalized epididymal fluid microenvironments promote numerous changes in the spermatozoa along the entire length of the epididymal tubule (von Horsten et al., 2007).

## 6.2 The epididymis and the protection of the mature spermatozoa

In humans, the spermatozoa take approximately ten days to travel from the initial segment to the cauda region of the epididymis (Toshimori, 2003). Once they are fully mature, the spermatozoa may be stored in the terminal region of the cauda epididymis for days or weeks, depending on the species, until ejaculation occurs (Yanagimachi, 1994). The mechanisms of sperm survival in the distal epididymis are poorly understood. The spermatozoa are at risk during transit and during the period of storage within the epididymis. The predominance of polyunsaturated fatty acids (PUFA) in the plasma membrane of the spermatozoa renders them highly susceptible to lipid peroxidation because of attacks by reactive oxygen species (ROS). The development of enzymatic and non-enzymatic strategies for protecting the spermatozoa during this extremely vulnerable period is another role attributed to the secretory activity of the epididymal epithelium. Some of the proteins released by the epididymal epithelium into the lumen seem to be involved in the protection of the spermatozoa from both oxidation reactions and/or bacterial attacks (Robaire et al., 2000; Gatti et al., 2004; Robaire et al., 2006).

A system for the regulated storage of spermatozoa in the distal region of the organ was developed in mammals, ensuring that the stored cells are quiescent and unreactive (Gatti et al., 2004). The exact mechanism of this physiological phenomenon, known as sperm quiescence, is not clearly understood. One hypothesis is that remaining in a quiescent state

protects the sperm from the drastic change in ionic composition of the medium in the cauda epididymis. Other factors, such as the inorganic and organic constituents of the luminal fluid, are of secondary importance and might assist in inducing sperm quiescence (Gatti et al., 2004).

Finally, there are a number of epididymal sperm-coating proteins that exert their effects on the male gamete in the female, and not in the male tract. These proteins produced by the epididymis and bind the luminal sperm, but they become functional in the female oviduct. Some of these molecules are considered to be decapacitating factors and might bind to the spermatozoa surface to prevent premature sperm activation (Kobayashi & Behringer, 2003).

### 6.3 Postepididymal events

After undergoing epididymal maturation, the mature spermatozoa are stored in the cauda epididymal segment until ejaculation. Once in the female reproductive tract, the mature spermatozoa must be capacitated. Capacitation is a complex maturational phenomenon that renders spermatozoa capable of binding and fusing with the oocyte, which is a requirement for mammalian fertilization. Capacitation encompasses plasma membrane reorganization, ion permeability regulation, cholesterol loss and changes in the phosphorylation state of many proteins (Visconti et al., 2011).

To acquire the potential for fusion with the oocyte, the capacitated spermatozoa initially interact in a species-specific manner with the zona pellucida, an extracellular coat that surrounds the mammalian egg. This process, called primary binding, is mediated by the zona pellucida glycoconjugates that recognize the sperm receptors located on the surface of the male gamete. These receptors are incorporated into the spermatozoa surface during epididymal maturation (Bedford, 2008; Robaire et al., 2006). The bound spermatozoa undergo the acrosome reaction and initiate penetration into the zona pellucida. Sperm penetration involves both the digestion of the zona pellucida and vigorous sperm motion. The sperm is also kept bound to the matrix via sperm receptors, an interaction that is called secondary binding (Flesch & Gadella, 2000). Numerous candidates have been postulated as possible sperm receptors for primary and secondary binding, such as human P34H, an epididymal sperm protein secreted predominantly by principal cells in the proximal-distal segment of the corpus epididymis (Boué & Sullivan, 1996).

Acrosome-mediated spermatozoa that have penetrated the zona pellucida enter the perivitelline space, bind and fuse to the egg plasma membrane and release the genetic material that will initiate zygote development. Protein complexes with both binding and fusion functions are present in both the egg and the spermatozoa and interact with their counterparts on the surface of the other gamete. Recent studies have demonstrated that the capacitated spermatozoa contain multiprotein complexes that are present on the sperm surface during capacitation. These complexes display an affinity for the zona pellucida in the event of fertilization (Toshimori, 2003). Several proteins of epididymal origin have also been proposed as participants in the sperm-egg membrane fusion process. In general, fusion proteins may have sequences of hydrophobic residues. They are mostly comprised of an alpha-helical structure and are known as fusion molecules. In a similar manner, the external proteins that are bound to the sperm surface reveal new functional peptide domains that may trigger the sperm-egg interactions such as protein fertilin  $\beta$  (also known as ADAM2)



that initially covers the entire testicular sperm head, is degraded by two successive cleavages during caput transit and is further restricted to the post-acrosomal domain of the spermatozoa (Toshimori, 2003).

## 7. The aging process of the epididymis

During aging, the male reproductive tract is characterized by testicular dysfunction resulting in the atrophy of the seminiferous epithelium and the Leydig cells. However, in rats, the average epididymal weight is not significantly affected by aging (3 months to 24 months). Aging affects the epididymis in a segment- and cell-dependent manner. Basal cells are primarily affected in the initial segment, clear cells are most modified in the caput segment and principal cells are most damaged in the corpus. In the proximal cauda segment, aging more dramatically affects the appearance of cells. In this segment, clear cells become enlarged and are filled with dense lysosomes, and some principal cells contain large vacuoles (Robaire et al., 2006).

Gene expression significantly decreases with age in the initial segment, the corpus and the cauda of the epididymis. The decrease in total epididymal gene expression from aging can be clearly observed in the corpus and the cauda, where expression of 83% and 62% of genes is respectively reduced to approximately 50%. This is in contrast to the initial segment, in which only 31% of the genes had a decrease in expression of at least 50%. The caput of the epididymis was the only segment in which the expression of large proportions of genes did not drastically change with age (less than 33%) (Robaire et al., 2006).

The function of the epididymis is highly dependent on the presence of androgens, particularly 5 $\alpha$ -reduced androgens. Rat models have shown that the ability of the epididymis to produce 5 $\alpha$ -reduced androgens becomes compromised with age. However the ability of the organ to respond to androgens is not compromised (Smithwick & Young, 2001).

Finally, the blood-epididymis barrier may be compromised in structure and function with age, resulting in the appearance of, and an increase in the number of, monocytes/macrophages mainly in the initial segment. Several kinematic parameters associated with sperm motility in the cauda of the epididymis may also be decreased. An increased incidence in the rates of pre-implantation loss, lower fetal weight and higher post-natal deaths was observed in the offspring of young female rats mated with older male rats (Robaire et al., 2000, 2006). Whether these effects are due to deficient epididymal maturation or due to a testicular dysfunction requires further study. Dysregulated intracellular trafficking, decreased protein degradation and oxidative stress are a few of the possible hypotheses that may explain the molecular mechanisms behind these changes (Robaire et al., 2000, 2006).

Many social factors have contributed to the increase in the number of men over the age of 35 who wish to become parents over the past several years. In 1970, fewer than 15% of all men fathering children were over the age of 35, but recent percentages have risen to almost 25%. Reproductive function gradually declines with advanced paternal age from multifactorial causes. Pattern quality parameters for fertility significantly decrease from 30-year-old men to 50-year-old men as follows: sperm motility ( $262 \pm 116$  ppm to  $110 \pm 152$  ppm), the volume



of semen ( $3.7 \pm 8.5$  mL to  $2.1 \pm 1.5$  mL) and the concentration of spermatozoa ( $76 \pm 55$  million/mL to  $59 \pm 57$  million/mL), respectively. Most evidence suggests that increased aging has negative effects on male fertility and some genetic risk for offspring, but the age at which the risk develops and the magnitude of the risk are poorly defined, as is the role of the epididymis in this process (Stewart & Kim, 2011).

## 8. Epididymis and infertility

Approximately 13-15% of couples will encounter fertility problems during their reproductive life. Factors involving males are responsible for infertility in approximately 20% of couples and are contributory in another 30-40% of couples. This means that factors involving males and infertility are implicated in more than 50% of the difficulties couples encounter when attempting pregnancy (Hamada et al., 2011). Diagnostic tools and therapies to treat female infertility are relatively well developed. However, many of the causes of male infertility are considered idiopathic (Sullivan, 2004).

The spermogram accurately assesses male fertility through an evaluation of semen quality, sperm concentration, motility and morphology (Hamada et al., 2011). The normal spermogram values were based on multi-centered population studies on fertile men. However, several men presenting normal spermogram values are diagnosed as idiopathically infertile. These men may present with post-testicular defects that result in the ejaculation of spermatozoa with a normal morphology but with a sub-optimal fertilization capacity (Sullivan, 2004). The epididymis could be particularly involved in a number of the pathophysiologies affecting sperm maturation in some of these cases of male infertility (Sullivan, 2004; Hamada et al., 2011).

### 8.1 The epididymis, xenobiotics and endocrine disruptors

Xenobiotics are substances that are foreign to an organism. Some of these substances can produce adverse effects or damage under specific conditions of use, such as when these substances are present in much higher concentrations than are usual. Through the influence of lifestyle, environmental factors or prenatal exposures to compounds, xenobiotics can act as endocrine disruptors that reduce testosterone synthesis and androgenic signaling (Smithwick & Young, 2001; Marty et al., 2003). As previously described, the epididymis depends on androgens for both the development and maintenance of the organ. The epididymis is therefore a potential target for the toxic effects of xenobiotics, which may then influence male fertility. Experimental models that block the action of androgen produce side effects equivalent to a chemical orchietomy. The formation of DHT was blocked in the epididymis by inhibiting the expression of genes involved in signal transduction, such as fatty acid and lipid metabolism and the regulation of ion and fluid transport. This inhibition affected sperm quality but did not result in the complete obstruction of fertility. The blocking of the actions of estrogen resulted in a dramatic reduction in the fluid uptake capacity of the epididymal tissue and caused infertility (Robaire et al., 2000).

Epididymal histopathological studies and sperm function tests have not been standard in the fields of drug development or in the assessment of toxicants. The effects of xenobiotics on the epididymis may often have been overlooked (Robaire et al., 2000). In addition to the damaging potential of xenobiotic substances, there are concerns about the effects of new

anti-neoplastic drugs and herbal products, chemicals and plastics (cyclophosphamide, bisphenol A, phthalates sulfonates, and plasticizer), cleaning agents (alkylphenols) and pesticides/fungicides (mainly organochlorine compounds).

## 8.2 Epididymal abnormalities in cryptorchidism

Epididymal anomalies have been more commonly associated with undescended, rather than descended, testes. Testicular maldescent, then, is commonly associated with epididymal anomalies. However, most epididymal abnormalities are not likely to have contributed to testicular maldescent (Elder, 1992; Han & Kang, 2002). Cryptorchidism is a disease in which the testes and the epididymis are retained in the inguinal tract and the seminiferous tubules become atrophic as a result of the increase in temperature, which does not favor spermatogenesis (Garcia et al., 2011). The incidence of cryptorchidism is 1-4% in human male neonates (Toppari et al., 2001). The cause of cryptorchidism is multifactorial, although possible causes and risk factors, such as endocrine disorders, anatomical abnormalities and environmental and genetic factors, can explain the etiology of this phenomenon (Nieschalag et al., 2000).

The damage caused by cryptorchidism is reflected in a reduced diameter of the duct and a reduction in the length of the epithelium within the duct, along with the absence of spermatozoa in the lumen of the duct itself (Arrotéia et al., 2005; Garcia et al., 2011) (Figure 8).

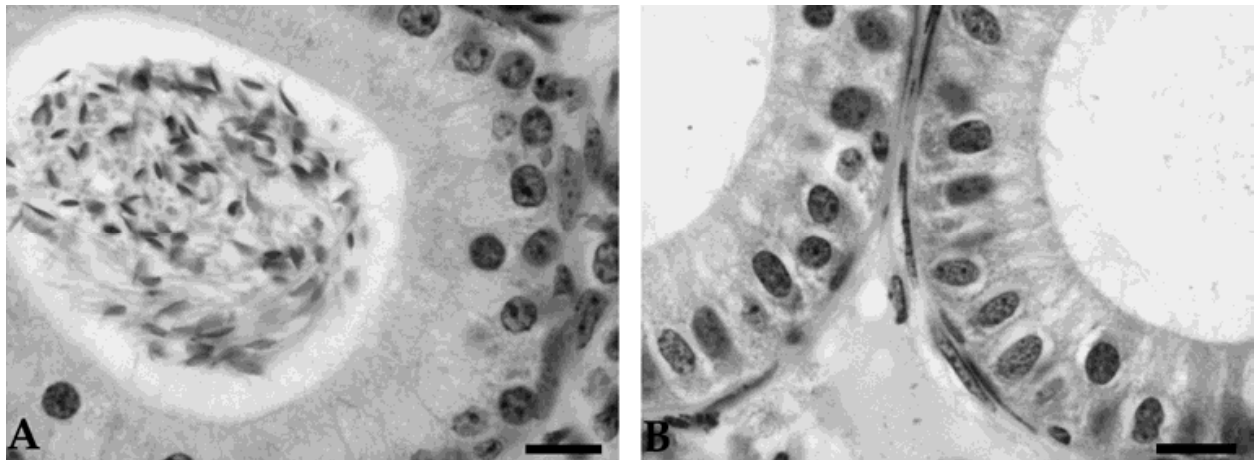


Fig. 8. Photomicrographs of a mouse epididymal caput. A: control photomicrograph of an adult epididymis; B: the epididymis of a cryptorchidic mouse. Sperm is absent because of the failure of production in the cryptorchidical testis. Scale bar: 50µm

Alterations in temperature also affect the ionic and protein composition of the cauda fluid, which effects the cauda epithelium by eliminating the special ability of the cauda to store and prolong the life of spermatozoa through the promotion of rapid epididymal transport (Nieschalag et al., 2000). In a series of studies on the epididymides of cryptorchidic animals and on primary epithelial cell cultures, it was shown that some epididymal gene products are exquisitely sensitive to small changes in temperature (Kirchhoff et al., 2000). Although there is no information on whether epididymal function (and hence sperm maturation) is irreversibly compromised in adult men who were submitted to orchidopexy as children, experimental results in mice indicate that gross and histological alterations caused by

cryptorchidism can be restored by orchidopexy (Arrotéia et al., 2005; Garcia et al., 2011). However, the decrease in the transit time of the spermatozoa in the epididymis (i.e., acceleration of sperm passage through the epididymal duct) and the reduction of fertility, fecundity and potency observed in cryptorchidic mice were not fully restored following orchidopexy (Garcia et al., 2011).

### 8.3 The expression of epididymal proteins and infertility

The fate of the proteins involved in spermatozoa maturation may interfere with successful fertilization. The proteins secreted along the epididymis under androgenic control vary from one segment to the other and modify the maturing male gamete in a sequential manner (Sullivan, 2004). The presence or absence of given molecules on the spermatozoa has often been correlated to certain traits of the sperm, such as the recognition and binding to the zona pellucida or oocyte membrane, or to the movement of the spermatozoa (Dacheux et al., 2005).

During the passage through the epididymal duct, molecules are incorporated into the development of the spermatozoa. Some molecules are more relevant than others in the maturation process and these or other molecules may play a relevant role in the acrosomal process later on. In the case of the protein P34H, which is related to male idiopathic infertility, the spermatozoa maturation process is a key event in the process of fertilization. Men diagnosed with idiopathic male infertility have an unexplained reduction in semen quality. These patients have no abnormal findings on physical examination and no laboratory abnormalities associated endocrine function. P34H is a 34 kDa human epididymal sperm protein synthesized and secreted predominantly by principal cells. P34H is physiologically undetectable on the spermatozoa in the caput of the epididymis and progressively accumulates on the sperm surface covering the acrosomal cap from the corpus to the distal cauda of the epididymis. This protein remains inactive in capacitated spermatozoa but apparently is lost during the acrosome reaction (Boué & Sullivan, 1996). This suggests that P34H is a human epididymal protein involved in the sperm interaction with the zona pellucida. P34H is associated with the spermatozoa of semen samples obtained from fertile donors, but it is undetectable in approximately 40% of the semen samples obtained from men presenting with idiopathic infertility. Spermatozoa with undetectable levels of P34H were related to the inability to bind to the zona pellucida through *in vitro* assays. These cases of infertility could be attributed to the inefficient epididymal maturation of male gametes that are then unable to fertilize. The human P34H protein and other epididymal molecules could be considered as markers for epididymal function in sperm maturation and can be used as a diagnostic tool to identify cases of infertility in men that have not been diagnosed through the classic methods of semen analysis (Boué & Sullivan, 1996).

Another possible protein interaction that may affect fertility is a protein complex recognized by the monoclonal antibody (mAb) TRA 54 (a high molecular mass albumin-containing protein complex) located in the acrosome of the spermatozoa that binds to the spermatozoa membrane during passage through the epididymal lumen. Experiments using *in vitro* fertilization have demonstrated that the addition of mAb TRA 54 to the fertilization medium significantly decreases the fertilization rate (Arrotéia et al., submitted for publication).

Similarly, the mAb 4A8 inhibited sperm penetration into the zona pellucida (Batova et al., 1998). Although the mAb used in these studies have the potential to recognize specific molecules that are directly involved in the formation of functional spermatozoa and form the molecular basis of gamete interaction in mammals, the recognition epitopes of these mAb are still not fully characterized (Arrotéia et al., 2004).

In addition to the causes indicated above, any disruption of the epididymal microenvironment through congenital abnormalities, intrinsic alterations in pH, protein composition and concentration, temperature, and other factors may lead to male post-testicular infertility. The study of the fate of molecules involved in sperm maturation and sperm-oocyte recognition represents a key to the etiology of the idiopathic male infertility.

## 9. Conclusion

The mammalian epididymis promotes the modifications of the spermatozoa that are necessary for the spermatozoa to become fertilization-competent cells and to be stored safely in the male reproductive tract. Since epididymal dysfunctions are related to cases of idiopathic male infertility, a focus on the major epididymal proteins related to the spermatozoa is important. From a clinical point of view, an increase in our understanding of spermatozoa maturation should provide the specific markers that will assist in the development of new criteria for both the prediction of male infertility and for improving the treatment of male infertility. The implementation of research from the fields of genomics and proteomics has assisted in the characterization of some of the already identified proteins, as well as in the description of novel epididymal components. We anticipate that these great advances will be helpful in the elucidation of the sperm maturation process.

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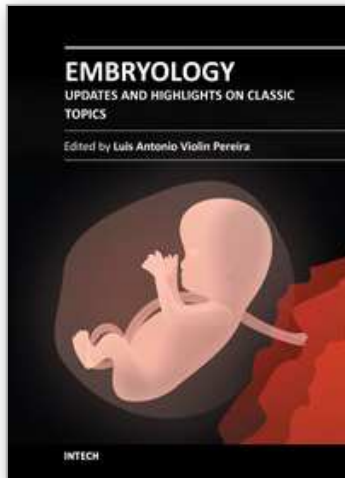
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