Chapter 18
What does the epididymis do and how does it do it?

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"If anyone asks what the epididymis is, we shall answer that it is a vessel constituting by various twists a body affixed to the back of the testicle" (de Graaf, 1668;see Jocelyn & Setchell, 1972).

Spermatozoa leaving the testis are neither motile nor able to recognize or fertilize an egg; they must traverse a long duct, the epididymis, to acquire these abilities. These post-testicular transformations of spermatozoa are collectively called sperm maturation. The epididymis is a single highly convoluted duct/tube of approximately 1 meter in length in the mouse, 3 meters in the rat, 6 meters in the human and a remarkable 18 meters in the stallion. Hence, it can take anywhere from 1 to 14 days for spermatozoa to traverse the epididymis. Early investigators considered the epididymis a holding tube for spermatozoa and that it was a place where spermatozoa aged. It was thought that the maturation process was inherent to spermatozoa and had little to do with the epididymis. It is now clear that the epididymis is very much an active participant in the maturation process by providing an appropriate luminal fluid microenvironment. The paternal genome being transported is in an ultracondensed and inactive form in epididymal spermatozoa; the acquisition of sperm fertilizing ability relies exclusively on molecules deriving from the testis (lumicrine factors) as well as from factors secreted by the surrouding epididymal epithelial cells. The challenge for many investigators has been to identify those molecules. In addition to its sperm maturational role, the epididymis creates an immune priviledge environment that protects spermatozoa against infection and immune attack as they mature (Chapter 20). It also provides an environment for maintaining mature spermatozoa stored in a quiescent state until ejaculation. Since spermatozoa are immotile, they require assistance to move along this very long duct. This movement is aided by contractions of smooth muscle cells that surround the duct as well

as pressure from fluid and spermatozoa entering the duct from the testis. From a clinical perspective, an malfunctioning epididymis results in male infertility, and therefore, the epididymis is considered to be a prime target for the development of a male contraceptive. Interestingly, unlike the testis and prostate, cancer is rarely observed in the epididymis.

Development of the epididymis

The intermediate mesoderm gives rise to the mesonephros, that in turn, gives rise to the mesonephric duct (Wolffian duct), Müllerian duct and mesonephric tubules. Under the influence of testosterone and anti-Müllerian hormone, the Müllerian duct degenerates leaving the Wolffian duct, which throughout development undergoes extensive elongation and coiling. The mechanisms by which the Wolffian duct elongates includes cell proliferation, cell intercalations, and biomechanical forces derived from the surrounding capsule and the extracellular matrix. Protein tyrosine kinase 7, inhibin βA, β-catenin, PLAG1, members of the sonic hedgehog and Hippo pathways, and SPAG11c, a β-defensin, are genes that have been shown to regulate elongation and coiling.

The gross anatomical structure of the epididymis in a variety of species is divided into several regions that include: the initial segment (observed in rodents), caput, corpus and cauda regions. Proximally, the efferent ducts connect the testis to the epididymis and distally, the vas deferens extends from the cauda region (Fig. 1). Within each region there are multiple segments separated by septa, with the numbers of segments within each region being variable. The challenge for investigators is to relate the different regions and segments to epididymal function and sperm maturation.

The epithelium of the epididymis is comprised of several cell types including: principal, basal, apical, halo, clear and narrow cells, each of which vary in number and size along the epididymal duct. For example, principal cells in the initial segment are tall resulting in a duct with a small luminal diameter whereas in the cauda region, the principal cells are low columnar and luminal diameter is much larger (Fig. 1). Through extensive analyses a much clearer picture is beginning to emerge regarding the function of each cell type within each epididymal region. Principal cells are known to actively secrete ions, organic solutes, proteins and transport water through aquaporin channels as a mechanism to concentrate spermatozoa.

Structure of the epididymis

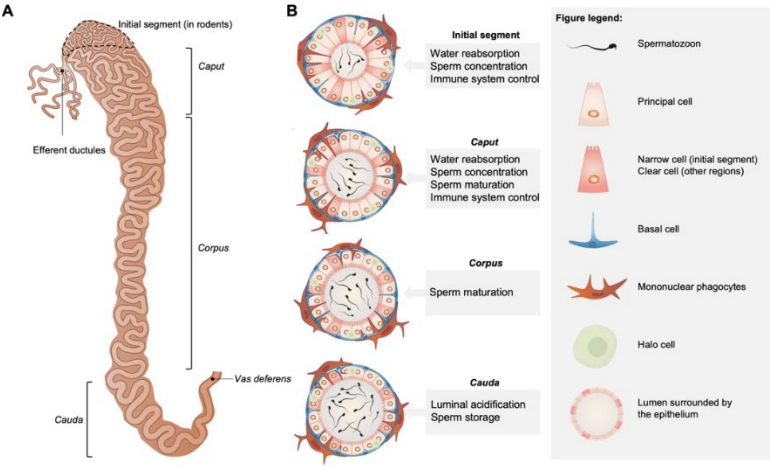

Figure 1. Structure, cellular organization and functions of the epididymis. (A) Schematic representation of the different regions of the epididymis: initial segment, caput, corpus and cauda. **(B)** Cross-sectional representations of the epididymal duct at each region. The major cell types and related functions are presented based on observations chiefly done in rodents. Adapted from Robaire B, Hinton BT, Orgebin-Crist M-C. The Epididymis. In: Neill, JD, ed. Physiology of Reproduction, Third Edition. New York: Elsevier. 2006; 1071-1148

They also participate in the release of extracellular vesicles following the formation of apical bleds from the apical membrane. These vesicles transfer bioactive molecules, including small non-coding RNAs and proteins, that participate in the control of sperm fertilizing ability and in the transmission of paternal traits acquired by environmental conditions to the offspring. Clear and narrow cells play a significant role in the acidification of the luminal fluid and also contain endocytotic machinery. Maintaining an acidic pH luminal fluid microenvironment is important for the maintenance of mature spermatozoa in a quiescent state. Basal cells display stem-cell like properties and form primary cilia as well as long axiopodia that sense and monitor the composition of the extracellular envrionment. The function of apical cells is unclear; however, there is evidence demonstrating that they endocytose material from the epididymal lumen. Halo cells are a mix of T lymphocytes, monocytes

and cytotoxic T-lymphocytes and may have a role in immune protection. Furthermore, a dense network of mononuclear phagocytes surrounds the epididymal epithelium and extends slender projections towards the lumen to control the immune environment. Surrounding the entire duct are layers of smooth muscle/myoid cells with the most numerous layers observed around the distal epididymis and vas deferens regions. Smooth muscle contractions aid the movement of spermatozoa and fluid along the epididymal duct. Single cell sequencing data performed on human and mouse tissues further extended the molecular and cellular complexity of the epididymis and will help improve our knowledge on the mecanisms controlling sperm maturation.

The blood-epididymis barrier

In view of there being a blood-testis barrier, it is not surprising to find a similar protective barrier throughout the epididymis. Physiological barriers perform several functions including providing a specialized luminal fluid microenvironment/milieu, protection against blood-born pathogens and xenobiotics, as well as providing immune-privilege. Classically, physiological barriers have been thought as being only the tight junctions between cells. It is now clear that barrier function is a complex interaction between the permeability properties of the basalateral and apical membranes, e.g. presence of channels and transporters, the permeability of the tight junctions themselves, i.e., the paracellular route, and any immune protective mechanisms provided in the lumen and the interstitial space. The blood- epididymis barrier is highly dynamic and its properties constantly change from the initial segment to the vas deferens. From a clinical perspective, the blood- epididymis barrier is a formidable hurdle to overcome when designing potential male contraceptive agents. However, small molecular weight novel male contraceptive agents could be designed that would be specifically transported into the epididymal cells/lumen by transporters located on the basolateral and apical membranes.

Animal models displaying epididymal and infertility phenotypes

Another challenge for investigators is to understand the role of secreted ions, organic solutes and proteins during sperm maturation. One approach addressing this challenge is to generate a

series of gene null mutations in mice that display an epididymal phenotype and infertility. The best known of the null mutations is Ros1 (c-Ros), an orphan tyrosine kinase receptor. Spermatozoa from these null animals display flagella angulation when exposed to the uterine, hypo-osmotic environment, rendering them incapable of reaching the egg for fertilization. Interestingly, the initial segment was found to be undeveloped in these animals, suggesting that the very proximal region of the epididymis is important for male fertility. Other murine models have also been found to display an angulated sperm defect and/or undeveloped initial segment, these include: GPX5Tag2, XXSry, "viable motheaten" (SHP-1 protein tyrosine phosphatase) null, Apolipoprotein E receptor 2 null, Acid sphingomyelinase null, Herc4 null, Dicer1 conditional knock-out, and Foxi1 null. Foxi1, a transcription factor, is of particular interest because it is known to regulate the expression of vacuolar H+-ATPase protion pump, carbonic hydrase II and the chloride/bicarbonate transporter found in narrow and clear cells. This null model provides clear evidence for the importance of the luminal fluid microenvironment during sperm maturation, changing the pH of the epididymal luminal fluid microenvironment in these animals resulted in male infertility.

Conclusion

In summary, the epididymis promotes sperm maturation, facilitates the transport of spermatozoa along the duct, stores spermatozoa and protect them from harmful substances. All of these functions are coordinated with remarkable precision to ensure production of fully viable, functional spermatozoa.

Suggested reading

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