

## 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 as 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, not only providing an appropriate luminal fluid microenvironment, but also supplying many of the molecules required by spermatozoa for the acquisition of fertility. The challenge for many investigators has been to identify those molecules. In addition to its sperm maturational role, the epididymis places a premium on protecting spermatozoa as they mature; it also provides an environment for storage following the maturation process. Since spermatozoa are immotile, they require assistance to move along this very long duct. This movement is aided by contractions of smooth muscle that surround the duct as well as pressure from fluid and spermatozoa entering the duct from the testis. From a clinical perspective, an improper functioning 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.

### Structure of the epididymis

The gross anatomical structure of the epididymis in a variety of species is divided into several regions that include: the initial segment, 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.

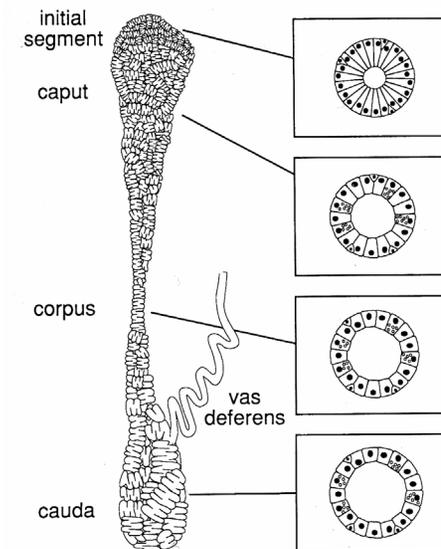


FIG. 1. Schematic representation of an epididymis showing the different regions: initial segment, caput, corpus and cauda. To the right are shown cross-sectional representations of the epididymal duct at each region. Note how the luminal diameter increases and the cell height decreases from the initial segment to the cauda.

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, 2). 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 and proteins. They are involved in endocytosis and many receptors and transporters are localized to their apical and plasma membranes. 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 sperm maturation.

Basal cells express a number of antioxidant proteins and are thought to play a role in protection from oxidative stress and xenobiotics. Halo cells are a mix of T lymphocytes, monocytes and cytotoxic T-lymphocytes and may have a role in immune protection. The function of apical cells is unclear; however, there is evidence demonstrating that they endocytose material from the epididymal lumen. Surrounding the entire duct are differing layers of smooth muscle/myoid cells (Fig. 2) 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.

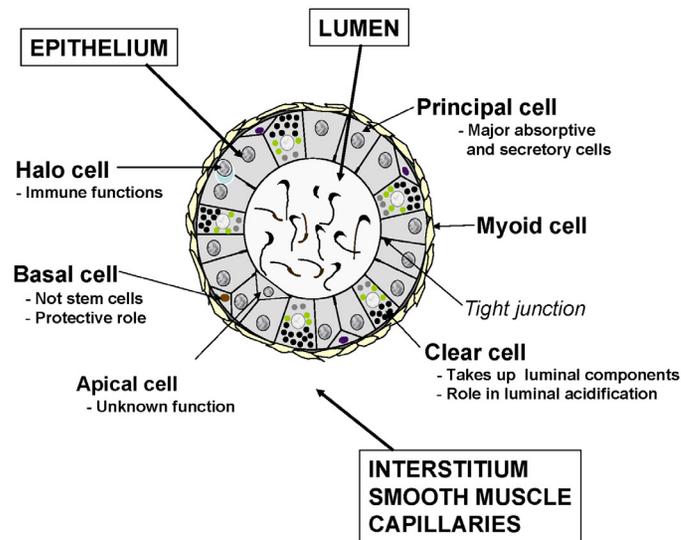


FIG. 2. Schematic organization of the major cell types in the epididymis as observed at the light microscope. The three epididymal compartments as well as the relative position and distribution of each of the main cell types are illustrated. The major function(s) associated with each cell type is also identified. 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.

### 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-borne 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 initial segment to 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 and *Foxi1* null. *Foxi1*, a transcription factor, is of particular interest because it is known to regulate the expression of vacuolar H<sup>+</sup>-ATPase proton pump, carbonic dehydrase 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.

In summary, the epididymis promotes sperm maturation, facilitates the transport of spermatozoa along the duct, stores spermatozoa and protects

them from harmful substances. All of these functions are coordinated with remarkable precision to ensure production of fully viable spermatozoa.

### **Suggested reading**

- Cooper TG, Yeung CH, Wagenfeld A, Nieschlag E, Poutanen M, Huhtaniemi I, Sipila P. Mouse models of infertility due to swollen spermatozoa. *Mol Cell Endocrinol* 2004; 216: 55-63.
- Jocelyn HD, Setchell BP. Regnier de Graaf on the human reproductive organs. *J Reprod Fert* 1972; Suppl. 17: 1-222.
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- Robaire B, Hinton BT. *The Epididymis. From Molecules to Clinical Practice*. Kluwer Academic/Plenum Publishers, New York. 2002.
- Turner TT. De Graaf's thread: the human epididymis. *J Androl* 2008; 29: 237-50.