

## Applying Electron Microscopy to Characterize the Human Epididymis Collected *in vivo*

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The epididymis is a long convoluted tubule that serves as a conduit for the transport of spermatozoa from the testis to the *vas deferens* and is the site where spermatozoa mature and acquire their functions of progressive motility and fertility. Unfortunately, very little is known about the human epididymis and its role in sperm maturation owing to the lack of studies conducted on normal human tissue. The role of epididymal luminal microenvironment and the mechanisms that epididymis uses to carry out its functions remain unsolved.

The first step to understand the processes required for sperm maturation in the human epididymis is to characterize the epididymal duct. Thus, in the present work the ultrastructure of the human proximal epididymis epithelium (Figure 1) and its specific secretory activity (Figure 2) were characterized using electron microscopy.

The human samples were collected *in vivo*, at the Centro Hospitalar de Coimbra, during a procedure aimed at collecting organs for transplantation from a healthy adult man at reproductive age. Then, the samples were processed for electron microscopy and visualized with a LEO 906 (Zeiss, Oberkochen, Germany) transmission electron microscope at the Institute for Anatomy and Cell Biology, Justus Liebig-University, Germany.

The ultrastructure analyses revealed that the human proximal epididymis is lined by an epithelium of ciliated and principal columnar cells (Figure 1). Additionally the ultrastructure analyses of the human epididymis, collected *in vivo*, revealed evidence of secretory activity in principal cells (Figure 2).

Our findings open new avenues for understanding the human epididymis physiology. The importance of understanding epididymal sperm maturation is emphasized by the fact that up to 40% of infertile men exhibit idiopathic infertility that may reflect sperm maturational disorders. Another important aspect is the potential to identify epididymal mechanisms that could serve as targets for non-steroidal-based male contraceptives.

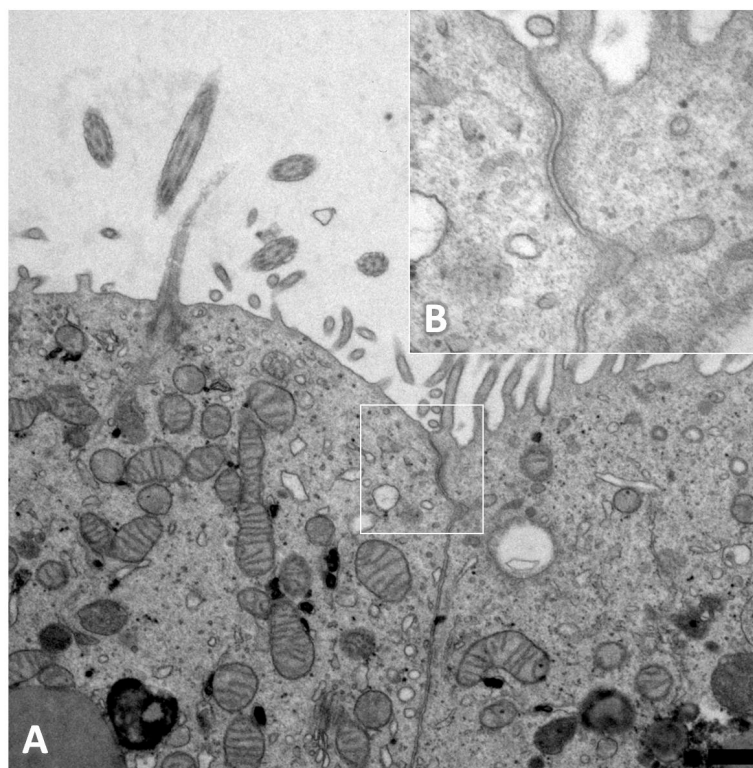


Figure 1. Human epididymis epithelium (TEM). A – cilia and microvilli projected from the luminal surface of the ciliated (left) and principal (right) cells into the ductular lumen. B (insert) – tight junction between the lateral plasma membrane of two adjacent cells (blood-epididymis-barrier). Scale bar, 500 nm.

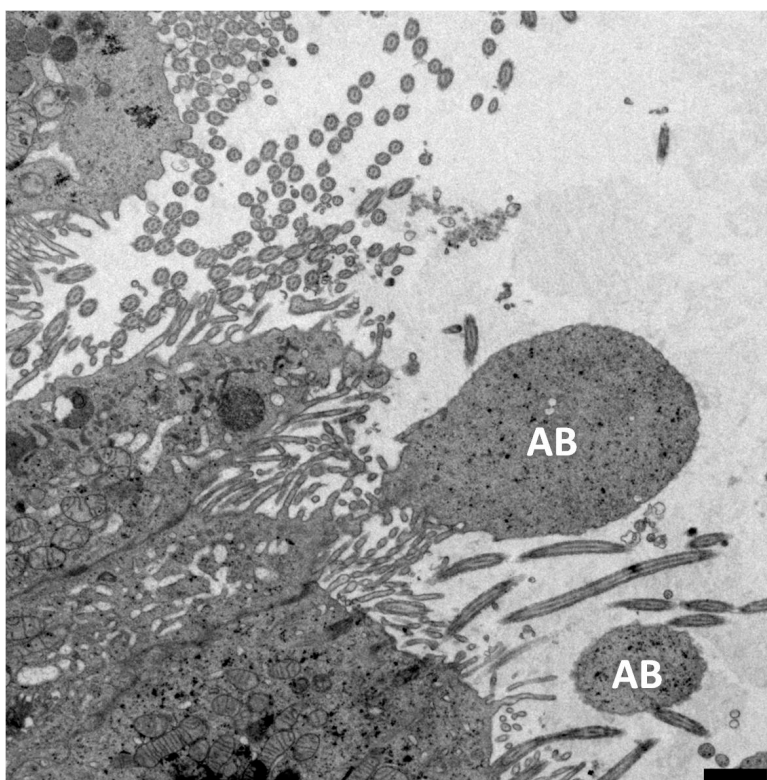


Figure 2. Human epididymis showing evidences of apocrine secretion in the principal cells (TEM). Apical protrusions (apical blebs, AB) detach from the principal cells and then breakdown to liberate their content (including epididymosomes) into the intraluminal compartment. Scale bar, 1  $\mu$ m.