In: Advances in Medicine and Biology ISBN: 978-1-53618-772-4
Editor: Leon V. Berhardt

Chapter 2

THE BLOOD-EPIDIDYMIS BARRIER: ITS RELEVANCE FOR MALE FERTILITY

Rita Payan-Carreira*
MED - Mediterranean Institute for Agriculture,
Environment and Development and Dept. of Veterinary Medicine,
ECT, Universidade de Évora [Pole at Mitra], Évora, Portugal

ABSTRACT

The existence of a blood-epididymis barrier is a core phenomenon in protecting the spermatozoa – that represent an immunologic challenge for the male organism - during their maturation and transit through the epididymides from an attack of the immune system. The blood epididymal barrier is less restrictive than the testicular one, and results from the colaboration of different cells and molecules that build up a protective shield and secure an immune privileged microenvironnement, vital for sperm maturation. In this chapter the three components that colaboratively engage in the blood epididymal barrier will be reviewed and their individual contribution to protection of the spermatozoa from the immune system discussed. Furthermore, it will also be critically discussed its relevance for sperm survival and male fertility.

* Corresponding Author's E-mail: rtpayan@uevora.pt or rtpayan@gmail.com.

Keywords: immune tolerance, epididymis, epididymal immunity, spermatozoa, blood-barriers, fertility

INTRODUCTION

Blood–tissue barriers work to protect key physiologic processes or cells from external cues, damaging molecules in the environment or another cells (Wen et al. 2018), generally related to immunologic processes, by restricting the access of both larger molecules and cells (whether those of the immune system or alien such as virus and bacteria) to the compartment they delimit. These barriers originate a specific milieu crucial to the optimal functioning of a particular tissue or structure.

There are several blood-tissues barriers in the body, namely the blood-brain barrier, the blood-retinal barrier, the blood-biliar barrier, the follicular barrier in female follicles or the blood-testis barrier, in the testicle, amonst others. In the male reproductive tract, blood cellular barriers protect the developing or maturing sperm, a haploid cell – in itself an immunologic challenge –, against the immune system. The most studied one is the blood-testis barrier, while the blood-epididymal barrier remained elusive for long (França et al. 2013). The blood-epididymis barrier (BEB) maintains a specialized luminal millieu for the maturing spermatozoa. By controlling the passage of a number of ions, solutes, and macromolecules across the epithelium (Robaire and Hinton 2015) and adapting the epididymal fluid to spermatozoa needs through its progress along the epididymal duct, BEB ensures the acquisition of forward motility and fertilizing ability (Avellar et al. 2019) hence contributing to male fertility.

Albeit the term “barrier” hints at a simplicity and uniformity of the structure and function, in fact, the body blood barriers are complex and extremely variable structures (Debbage and Thurner 2010), adjusting to the individual morphology and function of the organ and the cells needing protection and conferring it a particular protection. Most blood-tissue barriers are physically composed of tight junctions (TJ) established between adjacent endothelial or epithelial cells (Li, Mruk, and Cheng

2013, Wen et al. 2018), surrounding the stucture (organ or tissue) to protect, regulating the passage of cells into that space as well as regulating the transport of substances and fluids across the barrier, according to a directional flux. It is now accepted that in more complex barriers, other partners also intervene in the barrier constitution, either represented by a complexe interplay of different epithelial cell junctions, as it happens in the blood-testis barrier (Payan-Carreira and Santos 2020) or by the collaborative action of different cells and molecules, as in BEB.

THE BLOOD-EPIDIDYMIS BARRIER

The spermatozoon is a highly antigenic cell. It can easily trigger an immune reaction in the male reproductive tract if not protected behind a body barrier or some masking mechanism to disguise its immunoreactivity. The body needs to ensure tolerance toward spermatozoa during its production, epidydimal maturation, and storage, guaranteeing the viability of sperm cells (and thus male fertility), while retaining the ability to fight pathogens efficiently. The hematotesticular barrier physically isolates sperm cells from the immune system while in the seminiferous tubule. However, this protection is withdrawn as soon spermatozoa enter the excurrent male ducts. In these ducts, a similar but thin or incomplete barrier also exists (Witkin et al. 1996), namely in the epididymis.

The blood-epididymis barrier (BEB) constitutes the first line of defense in the epididymis. It is critical for sperm maturation during the epididymal transit, providing a specific controlled environment guaranteeing the protection and survival of spermatozoa. At the same time, it allows the acquisition of the necessary maturation and the acquisition of sperm fertility. In the epididymis, multiple epithelial cell types work concertedly to establish a safe, tolerant environment for the spermatozoa, to prevent autoimmune responses against auto-antigenic spermatozoa, while ensuring protection against ascending and blood pathogens.

General Morphology of the Mammal Epididymis

The epididymis is a single highly convoluted duct integrating the excurrent extratesticular excretory male genital ducts. It bridges the efferent ducts (ranging in number from 4 to 20) to the vas deferens (Robaire and Hinton 2015). It adheres to the lateral border of the testis (Figure 1); it is usually divided into three main regions: the caput, the corpus and cauda epididymides (Figure 1.A). In some species, such as the rodents and pigs, a distinct initial segment can be found between the efferent ducts and the epididymal ducts (Dyce, Sack, and Wensing 2010, Robaire and Hinton 2015).

The length of the epididymal ducts varies with the species, ranging from 1 m in mice to up to 80 m in horses (Robaire & Hinton 2015). The coiled ducts are densely packed and maintained in place by the surrounding loose connective tissue and a thick tunica albuginea, composed of dense connective tissue (Dyce, Sack, and Wensing 2010). Each epididymal region is arranged in lobules separated by connective septa (Figure 1.B), that contribute to the cohesion of the organ. The existence of perpendicularly oriented septa organises each region of the epididymis in lobules that help to maintain the epididymal form as well as it contributes to selective gene expression in each lobe (Turner et al. 2003, Sullivan et al. 2019). Such segmentation allows a specific region to act as a particular physiological compartment playing an essential role in the regulation of specific epididymal functions (Turner et al. 2003).

Externally, the epididymal ducts are delimited by a thin adventitia, that contributes to the interstitium of the epididymis. It surrounds a layer of circular smooth muscle which thickness gradually increases as the epididymis transitions to the deferens duct, as inner and outer longitudinal layers are added. Towards the interior of the tubule, a layer of myoid cells, arranged in multiple layers (in a species-specific number), surround the base membrane of the tubules (Krstić 1991, Robaire and Hinton 2015). The existence of epididymal myoid cells is not always recognised in the literature, and some studies include these cells (also named as myofibroblasts) in the smooth muscle coat (Elfgen et al. 2018). The inner

layer of the epididymal ducts represents the mucosa. It consists of a pseudostratified columnar epithelium containing different cell types. Some are found throughout the duct – as it is the case of the principal and basal cells – while others are specific or differently represented in particular regions of the epididymis – as it happens with the narrow or the clear cells (Robaire and Hinton 2015, Breton et al. 2016).

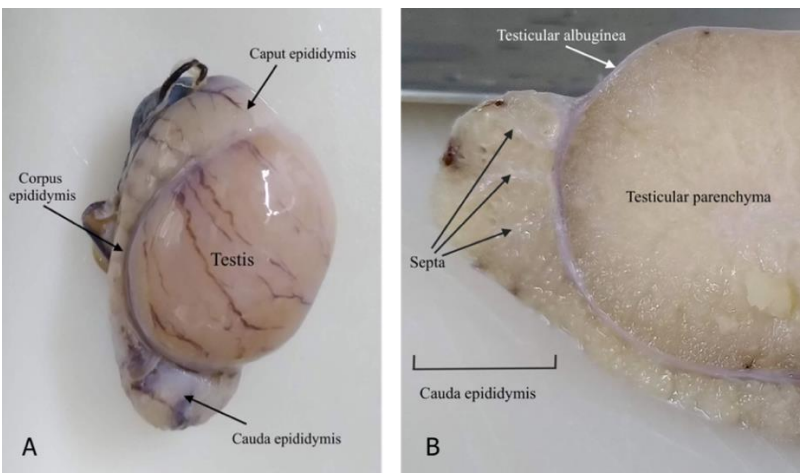

Figure 1. Canine testis and epididymis (formalin-fixed specimen). A – the epididymis runs in the lateral border of the testis and presents three main regions: the caput, corpus and cauda. B. on longitudinal sections, the existing septa originates smaller lobules representing different functional milieux adjusted to the sperm maturation needs.

Besides the differences in the smooth muscle layer, other variations have been found in the epididymal duct, namely in its diameter and the lumen, which becomes larger towards the cauda, in the epithelium height, which decreases from the head to the cauda, and the combination of epithelial cell types represented in there (Akbarsha 2016).

The principal cells are the primary cell type of the epididymal duct and have an important role in secretion and absorption. These tall columnar cells show basally aligned elongated nuclei, bear numerous very long, atypical microvilli, largely immotile (Figure 2 and 3) (Lowe and Anderson 2015). The principal cell microvilli increase 25-fold the surface exchange area between epithelial cells and the epididymal fluid, contributing to the

fast absorption of intraluminal fluid components at the epididymal brush border (Sullivan and Belleannée 2018). In the apical plasmalemma, principal cells form micropinocytotic vesicles (Krstić 1991), while in the apical pole, numerous vesicles (e.g., endosomes, lysosomes, and multivesicular bodies) can be found (Robaire and Hinton 2015, Akbarsha 2016). In the corpus region, the principal cells present numerous lipid droplets scattered through the cytoplasm (Robaire and Hinton 2015). The height of the epithelial cells shapes the height of the epididymal epithelium (Akbarsha 2016). It decreases towards the cauda of the epididymis, while they enlarge, assuming a more cuboid phenotype (Robaire and Hinton 2015, Akbarsha 2016). Principal cells are specialized in fluid secretion (Sullivan and Belleannée 2018). They also synthesize a large number of molecules (Robaire and Hinton 2015), including carnitine and phosphorylcholine (Krstić 1991, Sullivan and Belleannée 2018), essential for spermatozoa metabolism, as well as bicarbonate, whereby they participate in the acid/base equilibrium in the epididymis (Breton et al. 2016, Sullivan and Belleannée 2018). Principal cells also play a crucial role in the endocytic resorption of proteins present in the epididymal fluid (Robaire and Hinton 2015).

Basal cells are small round to prismatic shaped cells, representing the second-largest cell population found throughout the epididymis (Robaire and Hinton 2015, Akbarsha 2016). They usually locate beneath the principal cells but extend thin cellular projections towards the tubular lumen (Figure 2 and 3). It has been proposed that those projections – called axiopodia (Sullivan and Belleannée 2018) - may act as luminal sensors of the ionic composition of the epididymal fluid (Shum et al. 2008), used by basal cells to regulate the function of the epidydimal principal and clear cells (Touré 2019). Besides this sentinel role, basal cells actively participate in the oxidative stress mechanism in the epididymis, scavenging reactive oxygen species (Akbarsha 2016) and triggering the principal cells to secrete HCO3- into the lumen of the epididymal duct at ejaculation (Touré 2019). Basal cells are also involved in the regulation of electrolyte and water transport through principal cells, in a prostaglandin-mediated process, acting as humoral regulators of the principal cells (Akbarsha

2016). Studies from situations of obstructed epididymal sperm transit raised the hypothesis that basal cells may also be involved in the surveillance of sperm antigens (Akbarsha 2016).

Apical cells are mitochondria rich cells, localizing in the initial segment into the intermediate zone of the epididymis (Robaire and Hinton 2015, Akbarsha 2016). In these goblet-shaped cells, the ovoid nucleus is apically located, the luminal border is dome-shaped and presents several luminal flaps and folds (Figure 2 and 3), but no stereocilia/microvilli (Akbarsha 2016). These cells do not contact the basement membrane (Akbarsha 2016, Sullivan and Belleannée 2018). Aside from their ability to endocytose substances from the epididymal lumen (Robaire and Hinton 2015), the specific roles for apical cells remain poorly understood. It has been suggested that they may play a role in the inflammatory responses of epididymides, via the chemokine-modulated T-cell activation (Sullivan and Belleannée 2018). Also, evidence exists that these cells may also contribute to the control of the local oxidative stress balance (Akbarsha 2016).

Alike apical cells, the narrow cells are located exclusively in the initial segment or the intermediate zone of the epididymis (Robaire and Hinton 2015, Akbarsha 2016, Sullivan and Belleannée 2018). Slenderly elongated and narrow, they insinuate between adjacent principal cells in a way that only a small cellular projection reaches the basal membrane of the tubules (Figure 2 and 3), the larger proportion of the cell body locating into the luminal compartment of the tubules (Robaire and Hinton 2015). These cells show a deep-staining cytoplasm and dense, elongated nucleus located in the upper half of the cell (Robaire and Hinton 2015, Akbarsha 2016). Devoid of microvilli, they present small apical cup-shaped vesicles, multivesicular bodies and occasional endosomes and numerous lysosomes and mitochondria within the apical cytoplasm (Akbarsha 2016, Sullivan and Belleannée 2018). They have been proposed to participate in the acidification of the epididymal fluid in the initial segments of the epididymis (Sullivan and Belleannée 2018).

Clear cells, with their pale-staining cytoplasm, are found mainly in the corpus and cauda of the epididymis (Akbarsha 2016). These cells are

scarcely microvillated (Robaire and Hinton 2015, Akbarsha 2016), and also present numerous, relatively short membrane protrusions – microplicae (Figure 2 and 3) (Sullivan and Belleannée 2018). The apical pole of the cell is rich in mitochondria, multivesicular bodies, coated pits, endosomes and lysosomes (Robaire and Hinton 2015, Sullivan and Belleannée 2018). The endocytic activity is a strong role played by clear cells, particularly in the caputs region (Robaire and Hinton 2015, Akbarsha 2016). Through the epididymis, clear cells take up the contents of the sperm cytoplasmic droplets (Robaire and Hinton 2015, Sullivan and Belleannée 2018). These cells also contribute, together with the narrow cells, to the luminal acidification, as a mechanism to maintain spermatozoa quiescent before ejaculation (Sullivan and Belleannée 2018, Breton et al. 2016).

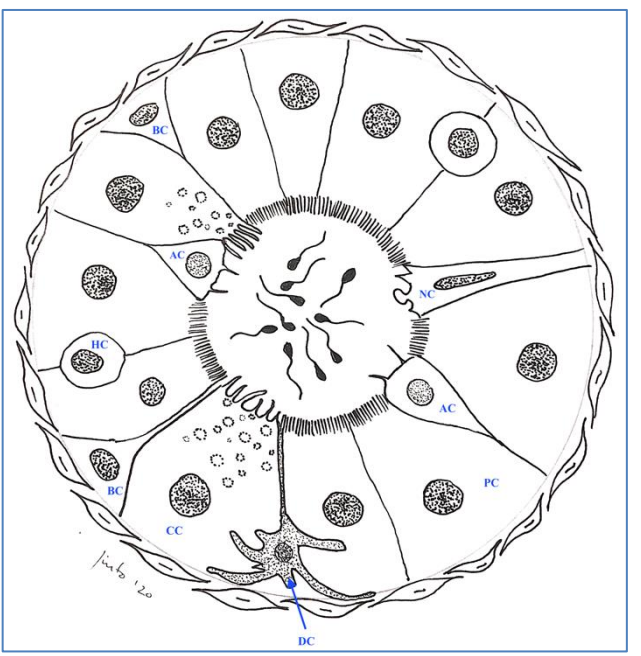

Figure 2. Graphic representation of the cellular elements of the epididymal epithelium. For the effect of the draw, the region of the epididymis (with dictates the cell type constitution) was ignored. PC – principal cells; BC – basal cells; CC – Clear cells; NC – Narrow cells; HC – Halo cells; AC – Apical cells; DC – Dendritic cells.

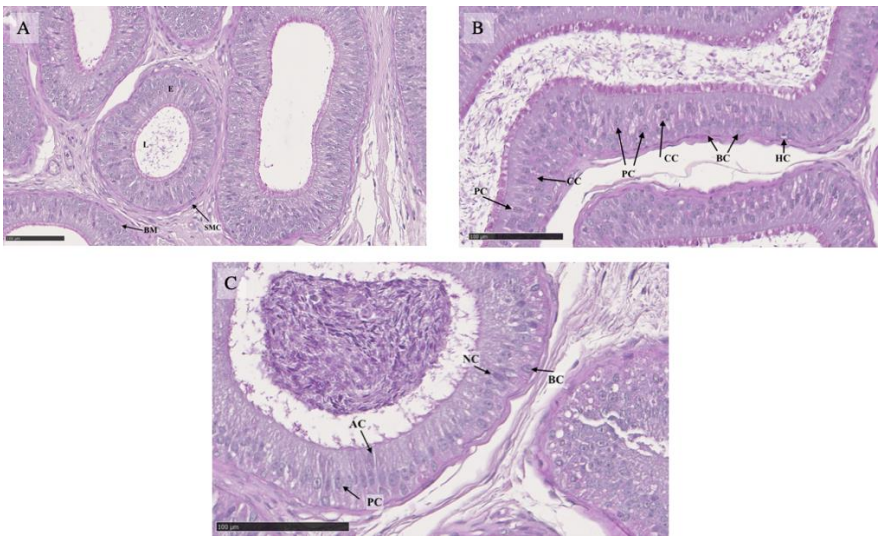

Figure 3. Dog epididymis (PAS staining). A - The edpididymal duct is lined by a pseudostratified epithelium (E), which is surounded by a basal lamina (BM) and a layer of sooth muscle cells (SMC). The lumen of the duct (L) is occupied by spermatozoa. B & C – A higher magnification shows that the epithelium is composed of different cells: PC – Principal cells; BC – Basal cells; CC – Clear cells; NC – Narrow cells; HC – Halo cells; AC – Apical cells.

Halo cells are small cells with a narrow border of clear cytoplasm (Robaire and Hinton 2015) found through the epididymis, albeit more abundantly represented in the proximal regions (Sullivan and Belleannée 2018). They are located at different heights along the epithelium (Akbarsha 2016), but are more often found in basal positions (Figure 2 and 3). Under this designation, several types of immunocompetent cells are represented, namely helper T lymphocytes, cytotoxic T lymphocytes, and macrophages. They are migratory cells (Akbarsha 2016), which number increases with ageing (Sullivan and Belleannée 2018). Despite the assumption that they are somehow involved in the epididymal immunoregulation, their exact functions remain unclear.

Dentritic cells have also been identified in the epididymal epithelium, where they form a dense network of cells extending long narrow dendrites between epithelial cells towards the lumen of the duct (Figure 2) (Hedger 2015, Sullivan and Belleannée 2018). They present specific features of

dendritic antigen-presenting cells in vitro, but their specific roles in epididymal immunological functions are still to be explored.

The Components of the BEB

Albeit the blood-epididymis barrier (BEB) shares some common features with the testicular blood-testis barrier (BTB), it is less restrictive, suggesting that the control of the immune system at the epididymis is different from other segments of the reproductive tract. This claim is supported by the fact that luminal infiltration of leucocytes may occur, and also immunoglobulins may access the epididymal luminal compartment.

BEB is a highly dynamic structure, always changing to meet with the needs of the maturing spermatozoa, according to the different epithelial cell types in the epididymis (França et al. 2013). The epidydimal duct architecture is a fundamental player in establishing the BEB and its protective effect against the immune system. Besides the type of cells, also the pattern of the epididymal junctional complexes and immune cells vary between segments, equally in number, length and organizational complexity (Dubé and Cyr 2013). This evidence suggests the need for different degrees of protection across the epididymis and distinctive environments adapted to the evolving maturational needs of spermatozoa.

Three components have been recognised to participate in the BEB (Mital, Hinton, and Dufour 2011):

1) The junctional complexes, which includes thigh junctions (TJs), combined with gap (GJs) and adherens junctions (AJs), constitutes an anatomic barrier that hampers the passage of molecules and cells across the epididymal epithelium;
2) The transporters system, that controls the movement of substances to and from the epididymal lumen producing a specialized microenvironment determinant for sperm maturation (physiological barrier);

3) An immunological component, that relies on non-immune (epithelial) cells that developed defense mechanisms as well as immune cells. It has been hypothesised that dendritic cells, an antigen-presenting cell population, localised between the epididymal cells, may be involved, together with T cells, in the establishment and maintenance of immune tolerance to maturing spermatozoa (França et al. 2013), suggesting that the complexity of BEB is far from be completely elucidated.

Alike in BTB (Payan-Carreira and Santos 2020), the junctional complexes established between neighboring epididymal cells are at the core of the BEB physical properties (Mital, Hinton, and Dufour 2011). Tight, adherens and gap junctions collaboratively maintain the fitness of the barrier limiting the traffic of molecules and cells from and to the lumen of the epididymis (França et al. 2013).

Tight junctions (TJs) between principal cells establish a continuous girdle in the apical area of the epididymal epithelium, dividing it into two compartments (the luminal and the basal compartment). In contrast, gap junctions ensure rapid communication between different epithelial cells (Robaire and Hinton 2015). Occludins and claudins are the main integral proteins identified in epididymal TJs (Dubé and Cyr 2013). In the adult male, occludin was identified in the apical area of adjacent principal cells; exception made to its association with narrow cells, which was only described in the initial proximal segment of mouse epididymis (Dubé and Cyr 2013). The levels of occludins and claudins vary greatly between the different epididymal regions (Gregory and Cyr 2006).

Different claudins have been identified in the epididymal epithelium, presenting a specific pattern according to the duct's regions, determining the regional specific permeability properties of the blood-epididymis barrier (Gregory and Cyr 2006, Dubé et al. 2010, Dubé and Cyr 2013). Some claudins form pores whose size varies with the claudin molecule, allowing the paracellular transport of ions across TJs (Cyr, Dufresne, and Gregory 2018).

As reviewed earlier, the epididymal epithelium presents multiple cell types that also establish connections with the principal cells. It has been proposed that cellular projections from narrow and dendritic cells may traverse the TJs complexes of adjacent principal cells. These cells establish tripartite intercellular junctions at the contact interfaces between three cells, which are mediated by tricellulin. Tricellular tight junctions (tTJs) seal the space between the three cells (Higashi and Miller 2017). Tricellulin has been identified in the apical area of the epithelium across the epididymis, in levels relatively similar between the different epididymal regions (Mandon and Cyr 2015). In most epididymal regions, tricellulin interacts with occludins and claudin-mediated TJs (Mandon and Cyr 2015). In the initial segment, tricellulin is expressed alone as well as overlapped with occludins, while these molecules colocalize in the apical area of the cells in all the other epididymal regions (Cyr, Dufresne, and Gregory 2018). Tricellulin-mediated junctions are important in modulating the epithelium permeability for ions, in particular, cations (Higashi and Chiba 2020).

Adherens junctions (AJs), bridging neighboring cells through cadherin-mediated pathways, serve structural and signaling functions (Cyr et al. 2002), providing further structure to the tight junctions mediated mechanism (Cyr et al. 2002). Besides participating in the barrier functions, AJs also participate in the control of the water movement, and the transfer of ions and proteins across the epithelium (Dubé and Cyr 2013). AJs are closely positioned to TJs. AJs involve two distinct adherence signaling pathways: the nectin-afadin and the cadherin-catenin complexes (Dubé and Cyr 2013). Both E- and P-cadherins were localized in the epididymis (Cyr, Dufresne, and Gregory 2018), the former being the most abundant. At the formation of the BEB, the E-cadherin complex bridges to the TJs complexes mediated by occluding via the β-catenin association with protein zona occludens (ZO)-1; it has been shown that cadherin-mediated AJs contribute to the initial formation of epididymal TJs (Cyr, Dufresne, and Gregory 2018). This association may be maintained through life, as it happens in the seminiferous tubules (Payan-Carreira and Santos 2020). This partnering between AJs and TJs is further supported by nectin-afadin

complexes that reinforce the interaction between the cadherin and occludin complexes (Dubé and Cyr 2013). Little is known about the nectin-afadin location across the epididymis. Whether the changes in the pattern of nectin incorporation into cadherin adhesive complex may explain differences in the adhesive properties of the epithelial cells and the barrier permeability in different epididymal regions deserves to be explored in the future.

AJs help maintain the epithelial shape and act as mechanosensors for the junctional tension in dynamic epithelia (Angulo-Urarte, van der Wal, and Huveneers 2020), for which they usually recruit vincullin into the cadherin complexes.

Besides cadherins and nectins, adherens junctions in the epididymis also enroll junctional adhesion molecules (JAM); these molecules further strengthen the interaction between adherens and tight junctions via occludins and some peripheral TJs proteins, such as the ZO proteins (Dubé and Cyr 2013).

AJs also participate in tricellular junctional complexes in cell vertices via the cadherin-catenin complexes that link to the actin cytoskeleton, in a pathway involving Sidekick proteins (Letizia et al. 2019). Albeit little is known about the structure and function of these junctions, it has been suggested tricellular adherens junctions (tAJs) may be of utmost importance during the mechanisms of cell intercalation, such as in the extension of cell projections from the basal compartment towards the lumen of the epididymal duct (Letizia et al. 2019, Angulo-Urarte, van der Wal, and Huveneers 2020), to maintain epithelial integrity in a remodeling situation. Besides the stabilization of the adherence between neighboring cells, tricellular cadherin-based junctions may also determine the channel size during paracellular permeability phenomena (Isasti-Sanchez, Münz-Zeise, and Luschnig 2020) in the epididymal epithelium, as well as in other epithelia.

The gap junctions (GJs) sustain rapid communication between cells and regulate the junction dynamics in mammals´ tissue barriers, providing essential crosstalk between the different types of junctions that co-exists at a blood-tissue barrier. In the epididymal epithelium, GJs are crucial

partners in the rapid paracrine regulation of the cell-to-cell signaling in the epididymis. GJs mediate the rapid intercellular transport of solutes via channels and allow for solute transport between the extracellular space and cells by creating GJs channels (Li, Mruk, and Cheng 2013). GJs consist of multiple pores created between cells, forming transmembrane channels through which the cytoplasm of adjacent cells contact (Cyr, Dufresne, and Gregory 2018). The bidirectional communication established between the cells is obtained by the passage of ions, small molecules (Cyr et al. 2002), secondary messengers, and small RNAs (Cyr, Dufresne, and Gregory 2018). Though GJs, cells are connected electrically and metabolically (Robaire and Hinton 2015).

In the epididymis, GJs are located between adjacent principal cells at their apical and lateral margins (Cyr et al. 2002), and between the principal and neighbouring cells. Connexins are the membrane proteins involved in GJs. Connexins may establish homotypic and heterotypic associations, allowing for a large number of possible combinations, since more than 21 connexins have been identified so far (Li, Mruk, and Cheng 2013, Cyr, Dufresne, and Gregory 2018). Different connexins own both unique and shared functions, which allow for partial compensation for the loss of one (Li, Mruk, and Cheng 2013). Six connexins combine according to a radial pattern– the connexon - to form a pore (Cyr 2011). The nature of the associated connexins determines the differences in charge and permeability of the intercellular pores, thereby explaining the selective permeability of GJs (Li, Mruk, and Cheng 2013, Cyr, Dufresne, and Gregory 2018). The activation and degradation of those combinations allow for a change in the spatio-temporal expression of connexins, and thereby rapid coordination of cellular events occurring within the epithelium.

Connexin 43 (Cx43) is one of the main connexins identified in the epididymal epithelium (Cyr et al. 2002, Cyr, Dufresne, and Gregory 2018). It is located mainly in the basal compartment, between principal and basal cells, as well as between the clear and basal cells and between neighboring myoid cells, particularly in the caudal epididymis (Cyr 2011). No Cx43 was found between adjacent principal cells, or between principal and clear cells (Cyr 2011). Impairment of Cx43 mediated GJs has been found to

have deep reflexes in sperm fertility parameters. Other connexins were identified in the epididymis [e.g., Cx26, present apically between neighboring principal cells; Cx31, mostly found in the epithelium of cauda epididymis; Cx32, that mainly connects adjacent principal cells; and Cx46 (Cyr et al. 2002, Cyr 2011)], hinting at a complex communication among epididymal cells (Robaire and Hinton 2015).

In several epithelia, connexins interact with TJs proteins, and it has been proposed that such interaction if fundamental to the anchorage of GJs plaques to the actin skeleton. Moreover, it has also been reported the existence of an interdependent assembling of GJs and AJs. The interaction of connexins with different junction proteins is tissue-dependent and aimed at the homeostasis of a complex blood-tissue barrier (Li, Mruk, and Cheng 2013).

PHYSIOLOGY OF THE BLOOD-EPIDIDYMIS BARRIER

The BEB presents two crucial roles regarding male fertility: it ensures the epididymal fluid matches the needs of the sperm throughout the maturation process, and it creates an immune-protected environment to the immunogenic sperm cell.

As spermatozoa traverse the epididymis, the unique epididymal microenvironment continuously changes, adapting to the particular needs of the maturing gametes. Along the epididymal duct, the luminal environment reflects the activity of the epithelial cells and the contribution of a highly dynamic blood-tissue barrier. BEB contributes to a physical fencing mechanism, preventing the spermatozoa contact with deleterious molecules and cells, and provides a physiological barrier controling the composition of the fluid, continuously and progressively changing across the epididymis to cope with the maturational needs of transiting cells (França et al. 2013). The creation of such an environment depends on the strict traficking control directed to a selective passage of ions, solutes and macromolecules through the epididymal epithelium.

Acting together, TJs, AJs and GJs along the duct contribute to the regulation of the highly specialized luminal microenvironment (Cyr et al. 2002), which results from the combined secretory and absorptive activities of the epididymal epithelial cells (Zhou et al. 2018).

The epithelial junctional complexes colaborates in the the selective transport of ions and solutes across the epithelium (Robaire and Hinton 2015, Anderson 2001), which occurs either by transcellular and paracellular transportation routes (Figure 4). The transcellular pathway is an active transport mechanism, highly specific and tightly regulated. It is directional, energy-dependent, and managed by a specific profile of transporters and channels positioned on the apical and basolateral cell membranes (Anderson 2001). Contrastingly, the paracellular pathway is involved in the passive transport of molecules through the epithelium using the intercellular spaces between epithelial cells (Rajasekaran, Beyenbach, and Rajasekaran 2008). It results from diffusion, electrodiffusion, or osmosis mechanims driven by the gradients created by the transcellular pathway and does not presents directional discrimination (Anderson 2001). Any junctional complexes concentrate and immobilize membrane proteins for extended periods of time, thereby raising impenetrable obstacles within the paracelullar spaces (Trimble and Grinstein 2015).

TJs are crucial in the transepithelial solutes and proteins trafficking. They play a double role: as a fence, they maintain the cell polarity and the boundary between the apical and basolateral membranes, blocking the membrane proteins movements from one membrane domain to the other (Breton, Nair, and Battistone 2019); as a gate, they seal the paracellular space and restrict the solute permeability (Breton, Nair, and Battistone 2019), controling the transepithelial ionic diffusion potentials through the activity of Na,K-ATPase, ions transporters and chanels (Rajasekaran, Beyenbach, and Rajasekaran 2008). The control of Na,K-ATPase activity further allows the TJs to interact with the AJs complexes indirectly, via the RhoA-mediated polymerization of actin and the stabilization of cadherin-mediated complexes as well as via ZO-proteins and JAM molecules. AJs are determinant in the assembly/disassembly process of TJs. Furthermore, the K^+-ATPase channels, known as regulators of epithelial tight junction

permeability, also indirectly regulate GJs permeability, via the Kir6/SUR complexes (Rajasekaran, Beyenbach, and Rajasekaran 2008).

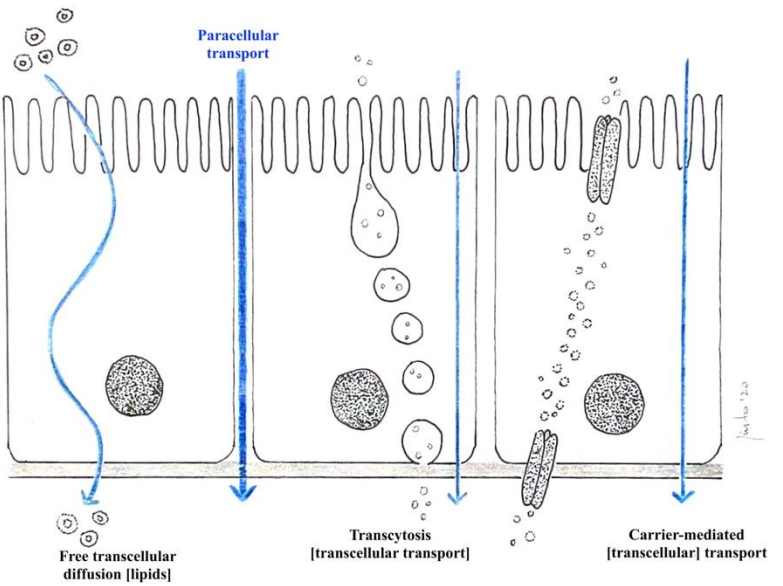

Figure 4. Transepithelial transport pathways (graphic representation). The transcellular route includes three different mechanisms, namely the freetranscellular difusion, usually for the transport of lipids, transocytosis (vesicle mediated transport) and the carrier-mediated transport, for water, ions and specific molecules. The paracellular route is a passive transport mediated by gradients and occurs between cells, controled by the intercellular junctions. MB – basal membrane of the duct.

To maintain spermatozoa quiescent while stored in the epididymis, it is essential to establish a low bicarbonate ($HCO3^-$) concentration and an acidic pH (Shum et al. 2011, Breton et al. 2016). The acidic epididymal pH is determined by a low level of sodium, chloride and bicarbonate ions (Touré 2019). Luminal acidification involves, among other processes, a balance between bicarbonate reabsorption by principal cells and its secretion (Breton et al. 2016). In the inicial and cranial segments of the epididymis resorption prevails over secretion, the later gaining importance in the more distal epididymal regions and vas deferens (Shum et al. 2009, Shum et al. 2011, Breton et al. 2016). Bicarbonate absortion is controled by the TJs, through the transcellular transport pathway (Shum et al. 2011,

Dacheux and Dacheux 2014). On the other hand, bicarbonate secretion is dependent on the dual activity of both the clear and principal cells (Park et al. 2017). In clear cells, bicarbonate resorption occurs by activation of V-ATPase-dependent proton-pumping, in response to changes in luminal pH. In principal cells, bicarbonate secretion occurs via the cystic fibrosis transmembrane regulator (CFTR) pathway, in a NHE3-dependent proton mechanism that endorses the secretion of $HCO3^{-}$ in activated cells, and the proton secretion under cellular resting conditions (Park et al. 2017, Touré 2019). In the mechanims contributing to an acidic luminal environment, the ions and water movements across the epithelial cells are dependent of a intimate crosstalk between clear cells, basal cells and principal cells, a mechanism that ulterly benefits from GJs. Basal cells have the ability to modulate V-ATPase in clear cells, triggering the secretion of H^{+} into the epididymal lumen. In principal cells, anion secretion is driven via bradykinin secreted by basal cells (Shum et al. 2011). Bradykinin activates de prostaglandin E secretion, which in turn activates the CFTR channel in principal cells, and the secretion of anions and water into the epididymal duct (Park et al. 2017, Breton, Nair, and Battistone 2019).

Moreover, the BEB is a critical player at the interface between the immune system and fertility. While traversing the epididymis, sperm antigens have the potential to trigger an immune response. This ability could compromise spermatozoa viability. Therefore, in a mechanism endorsing male fertility, BEB contributes to the spermatozoon's immunotolerance during its maturation and storage in the epididymis (Voisin et al. 2019).

To descriminate between self and foreign antigens, the epididymis relies on a dual mechanism, of both central and peripheral tolerance. The central mechanism is established way before puberty; the peripheral tolerance mechanism allows for a local modulation of the central mechanism, that develops after puberty to protect the spermatozoa.

It is well accepted that the epididymal epithelium is the prime actor in the immune surveillance system to protect the male gametes. It acts as an immunological barrier composed of non-immune cells that developed a defense mechanism and of cells of the immune system. Both cell types

respond to specific attacks and contribute to sperm tolerance. Nevertheless, the role of BEB in the establishing of epididymal immune tolerance remains poorly understood.

Some epididymal epithelium cells develop a defense mechanism, reacting against ascending or blood-born pathogen invasion, and maintaining a pathogen-free environment in the ducts (Voisin et al. 2019). Toll-like receptors expression, known to recognize pathogen-related molecular patterns, was detected in the principal cells, mainly in the caput and corpus epididymis (Hedger 2011), together with inflammatory sensor molecules, such as cytokines and interferons (Voisin et al. 2019). β-defensins, a large family of proteins presenting a broad-spectrum antimicrobial activity, dependent on cationic amino acids, were also demonstrated in the epididymis (Hall et al. 2007, Ribeiro et al. 2016). In general, they are abundantly expressed through the epididymal epithelium in a spatially and temporally regulated pattern (Hedger 2015, Ribeiro et al. 2016). They are also secreted into the fluid, usually combined with different defensin molecules, and have been evidenced at the sperm surface (Ribeiro et al. 2016), particularly when stored in the cauda regions (Hall et al. 2007). It has been proposed that different β-defensins act synergistically and sequentially in the epididymal luminal fluid, thereby collaborating to sperm protection, maturation and fertilization ability along the epididymal duct (Zhang et al. 2011). Furthermore, at the spermatozoon level, they have been proposed to modulate the acquisition and maintenance of motility by controlling the intracellular calcium and regulating the acrosome reaction after ejaculation (Ribeiro et al. 2016). Besides, defensins act as immunoregulators in the inflammatory response mediated by LPS, possibly interfering with the Toll signaling pathways, and they are chemotactic for immature dendritic cells and T lymphocytes (Voisin et al. 2019). Additional immune-regulatory molecules were detected in the epididymal epithelium, such as lactoferrin, interleukins and chemokines (Voisin et al. 2019), that will not be addressed in here. Some of those molecules could divert the local immune system towards an anti-inflammatory profile (Voisin et al. 2019).

Besides protecting the epididymis against the invasion of pathogenic microorganisms, the epithelium prevents an autoimmune response against autoantigenic spermatozoa. In the epididymis, B and T cell populations - located mainly in the interstitium and sporadically between epithelial cells [where they are collectively named as halo cells (Hedger 2015)]-, macrophages and dendritic cells cooperate for adaptive cellular immunity. All of these cells are absent of the lumen of the duct in physiological conditions (Guiton et al. 2019, Voisin et al. 2019).

Dendritic cells are organized as a dense network in the basal compartment of the epididymal epithelium (Da Silva et al. 2011). They extend dendrites toward the lumen between epithelial cells (Voisin et al. 2018), acting as "sentinels" of the immune system (Da Silva et al. 2011). Dendritic cells are stellate, specialized cells with a robust antigen-presenting ability (Da Silva et al. 2011); they are particularly abundant in the initial segments of the epididymis. It has been hypothesized that they may likely play a primary role in the acceptance of maturing sperm cells by the immune system (Da Silva et al. 2011, Da Silva and Barton 2016). Dendritic cells interact with the local T lymphocytes, in particular with the CD4+ subset of T cells (Hedger 2015, Guiton et al. 2019), via the induction of regulatory T lymphocytes (Da Silva and Barton 2016) or the indolamine-induced inhibition of T lymphocytes growth (Guiton, Henry-Berger, and Drevet 2013, Da Silva and Barton 2016, Voisin et al. 2018). This effect foster the creation of a local tolerance towards the specific antigen combination, newly exposed in maturing spermatozoa, and contributes to the establishment of the necessary selective local immune tolerant state. The fact that indoleamine is constitutively expressed in the epididymis suggests that this organ is normally in a particular state of anti-inflammatory response (Guiton, Henry-Berger, and Drevet 2013).

Macrophages are more frequently identified in the interstitium and the peritubular area than in the epithelium (Hedger 2011, Guiton et al. 2019). Their number increases with age in the caput and corpus, but not in the cauda epididymis (Hedger 2015, Guiton et al. 2019). Macrophages are associated to the epididymal homeostasis because they engulf apoptotic

cells and clean debris from the lumen in cooperation with the surrounding epithelial cells (Da Silva and Barton 2016).

REGULATION OF THE BLOOD-EPIDIDYMIS BARRIER

It has been demonstrated that the regulation of the blood-epididymis barrier is complex, multifactorial, and simultaneously segment- and age-specific (Dubé and Cyr 2013). Thereby, we only tackle a small part of it, as it may be needed for the following section of this review. The focus will be put on androgens, the retinoic acid and TGFβ pathways as important modulators of the BEB (Robaire and Hinton 2015).

The formation and maintenance of BEB is androgen-dependant. Testosterone is converted in dihydrotestosterone upon its entry in the epididimis (Ezer and Robaire 2002, Dubé et al. 2010); both testosterone and dihydrotestosterone bind to the androgen receptor. The androgen receptor has been identified in the epididymal epithelial cells and in the smooth muscle cells. When activated, it modulates both the BEB structure and gene expression of multiple molecules related with the local immune tolerance. Principal cells are the most sensitive to androgen influences (Robaire and Hinton 2015). In the absence of androgens, the height of the epithelium decreases, the principal cells loose their apical microvilli, and the secretion apparatus decreases in importance (Ezer and Robaire 2002, Britan 2006, Robaire and Hinton 2015). These changes, along with a reduction of the total protein and RNA content (Robaire and Hinton 2015), suggest that androgens affect the secretory ability of the epididymal epithelium, thereby affecting the maturation process of spermatozoa. Androgens also control the transport of ions and small molecules and the selective absorption of luminal content (Ezer and Robaire 2002), and regulate the integrity of BEB by modulating the claudins turnover (Dubé et al. 2010). They also modulate both the apoptosis and proliferation in the epithelial cells (Britan 2006). In the normal postpubertal physiological state, androgen-dependent anti-proliferative signals inhibit cellular proliferative capacity (Ezer and Robaire 2002, Robaire and Hinton 2015).

Also, they were associated with the control of epididymal β-defensin expression in multiple species (Ribeiro et al. 2016).

Estrogens have been associated with the control of ion transport and with the development and maintenance of the BEB structure, particularly mediated by clear and apical cells (Britan 2006). They are also associated with the poliferation activity in the epididymal cells before puberty. It has been proposed that spermatozoa transiting the epididymis can convert androgens into estrogens that will enter the epithelial cells and trigger the secretion proteins into the lumen, allowing to mach it with the needs from sperm maturation (Hess, Zhou, and Nie 2002).

Retinoids, the vitamin A derivatives, are necessary for the integrity of a differenciated epithelium, and also participate in the synthesis and secretion of different epididymal proteins (Britan 2006). The cellular retinol binding proteins (CRBP) have been evidenced in the epididymal cells, according to a species-specific pattern (Orgebin-Crist et al. 2002). Dendritic cells metabolize vitamin A into retinoic acid (Guiton, Henry-Berger, and Drevet 2013). In the absence of retinoids, numerous epithelia develop squamous metaplasia (Orgebin-Crist et al. 2002). In the epididymis, retinoids affect the synthesis of several proteins, in an androgen-independent manner (Astraudo et al. 1995). Impaired reticnoic acid signaling results in squamous metaplasia of the epididymal epthelium, but also in an increased number of macrophages infiltrating the epididymis, triggering a mild inflammatory response (Jauregui et al. 2018). The epididymal Retinoic Acid Binding Protein (E-RABP) or lipocallin is one of the major epididymal secreted proteins (Ong et al. 2000, Orgebin-Crist et al. 2002). It possibly works as a transporter for retinoic acid synthetized in the epithelium. The observed decrease in retinyl ester in spermatozoa transiting through the epididymis drove the hypothesis that this molecule may be converted into retinoic acid, which binds to E-RABP (Ong et al. 2000). In other epithelia, retinoic acid has been shown to influence lymphocyte migration and immunoglobulins traficking (Guiton, Henry-Berger, and Drevet 2013), which in the epididymis may contribute to the age-associated decline in fertility.

TGFβ is present in high values in the epididymes, hinting that it may have important roles to play. The TGFβ has been involved in the regulation of epithelia permeability, which may be partly mediated by quantitative changes in tight junctions (Stammler et al. 2013). TGFβ increases the paracellular permeability and also modulates the claudin activity and the paracellular tightness (Stammler et al. 2013). Besides TGFβ has an important role in immunoregulation. TGFβ is crutial in the creation of peripheral immunotolerance (Voisin et al. 2020). Pierucci-Alves et al. (2018) showed that TGFβ signals dendritic cells to establish the local immunotolerance to sperm located in the epididymis. Modulation of the BEB permeability and the tolerogenic role of TGFβ may be interconnected, since the changes in paracellular permeability would also facilitate the migration of dendritic cells into the epididymal epithelium (Voisin et al. 2020).

LOSS OF FUNCTION AT THE BLOOD-EPIDIDYMIS BARRIER

The BEB loss of function can configure different situations; the most frequent are ageing, the obstructive disorders, and the traumatic and inflammatory conditions of the scrotum or epididymis (Dubé and Cyr 2013). Any one of these conditions may lead to the production of antibodies directed to spermatozoa which in turn will lead to male infertility (Figure 5). In humans, it has been recently reported that epididymal diseases, more than the testicular´s, are associated with the occurrence of antisperm antibodies (Lotti et al. 2018), even in the idiopathic infertility. Any dysfunction of the BEB efficiency will impair male fertility. Up to 15% of male infertility has an immunological origin, either due to repetitive infections or to autoimmune responses. Nevertheless, despite its importance, the study of the epididymal immune tolerance system and BEB remains less explored than that of the testis.

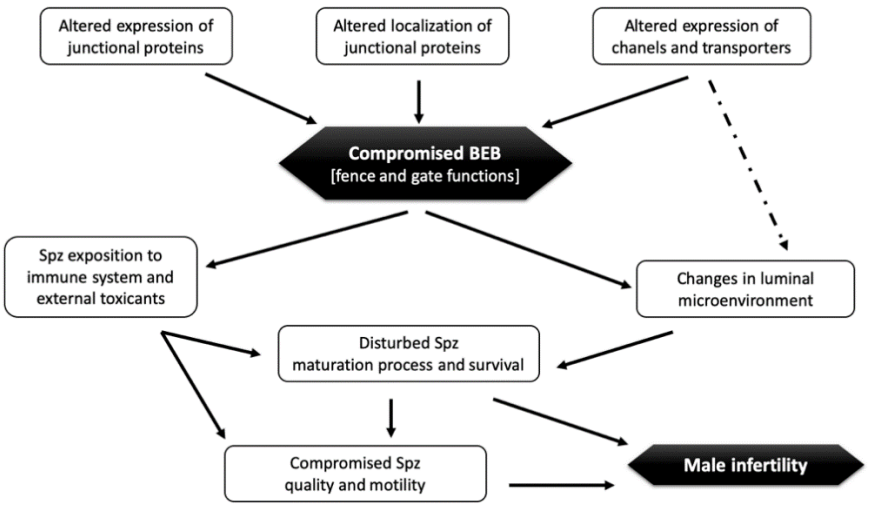

Figure 5. contributions of the blood-epididymal barrier (BEB) in spermatozoa (Spz) function and male infertility (adapted from Dubé and Cyr (2013).

With ageing, the number of halo cells in the epididymis increases in a segment-specific pattern (Serre and Robaire 1999, Cyr et al. 2007), that accompanies a reduction on the number of spermatozoa in the epididimal lumen and the accumulation of proteinaceous luminal debris (Robaire 2002). Besides the increased number of halo cells, it is also observed a reduction in proportion of principal cells and basal cells through the epididymis, as well as in the relative number of narrow cells in the initial segment and of clear cells in the corpus epididymidis (Robaire 2002). With increasing age, an increased number of inflammatory cells infiltrating the interstitium has been reported (Robaire 2002), albeit the differences in their number are hardy significant compared to the increased recruitments of immune cells into the epithelium and lumen of the ducts (Serre and Robaire 1999). Besides changes in the number of cells infiltrating the epipidymal epithelium, it was also observed an age-related alteration of the ultrastructure of principal cells and some clear cells, more pronounced at the cauda region (Serre and Robaire 1999), co-existing with the thickening of the basement membranes (Serre and Robaire 1999, Robaire 2002).

It has been proposed that with age, the constitution of the epididymal fluid may change, leading to the accumulation of abnormal or foreign

substrates that drives the recruitment of an increasing number of immune cells within the epithelium (Serre and Robaire 1999). Furthermore, age-related, segment-specific changes were reported in the localization and expression of occludin and other TJs proteins and cadherins, which could contribute to the barrier dysfunction (Dubé and Cyr 2013, Bosveld, Wang, and Bellaïche 2018) and the changes noticed in sperm motility (Dubé and Cyr 2013). With age, a reduction in occludins and ZO proteins was described, suggesting a decrease in the TJs tightness between adjacent cells (Robaire 2002) that would contribute to an altered permeability of the epididymal epithelium.

Ageing has also been associated with increased oxidative damage of epididymal epithelium (Noblanc, Klaassen, and Robaire 2020) and an increase in the apoptosis rate of epididymis epithelial cells (Bosveld, Wang, and Bellaïche 2018). Some of the oxidative stress enzymes in principal cells are androgen-depend. The age-reduction in androgens synthesis migh explain the failure to control the local oxidative damage (Ezer and Robaire 2002). These changes are similar to the observed after castration, leading to the assumption that they may be trigggered by an imbalance in androgens secretion (Elzanaty 2007).

Epididymal obstruction may occur secondary to vasectomy, segmental agenesis of the cauda epididymis or deferent duct, or develop as a sequela for chronic inflammation originating granulomas. Epididymal obstruction drives altered sperm transport (Kirchhoff 2018). The upstream accumulation of fluid (and sperm stasis) increases the intraductal pressure, alters the fluid pH and composition and hampers the sperm maturation process. The severity of the loss depends on the local of obstruction (Dube et al. 2010). A gradual decrease in the duct diameter occurs at sites of luminal distension (Aktuğ et al. 2006, Pal et al. 2006, Sarda et al. 2011), along with flattening of the epididymal epithelium and the loss of microvilli in a local or diffuse pattern (Sarda et al. 2011). The few remaining apical microvilli become markedly reduced in size (Pal et al. 2006). The epithelial cells present pigment that putative represents remnants of the spermatozoa ingestion (Sarda et al. 2011). In parallel, the intertubular connective tissue increases (Pal et al. 2006, Dube et al. 2010),

and infiltrates with a larger number of lymphocytes, plasm cells and macrophages (Sarda et al. 2011). The increased number in local inflammatory cells has been associated with sperm extravasation in the interstitium, and with increasing amounts of antisperm antibodies. These observations suggest the disrupted BEB, due to TJs and AJs damage, affects the paracellular transport (Dube et al. 2010), dysregulating fluid reabsorbtion (Kirchhoff 2018), as well as facilitating the traffick of immune cells and spermatozoa across the epithelium. With time, obstructive disorders of the epididymis originate a local latent chronic inflammatory condition.

The epididymis is a target for ascending or blood-born infections. Prevalence of infections in the male reproductive tract varies with the species, but may account for approximately 15% of men infertility (Dubé and Cyr 2013). The most common conditions include interstitial edema and inflammatory infiltrate of the epididymis, and granuloma formation, with subsequent sperm stasis. But the inflammation of the epididymis may also occur in response to a drug, or secondarily to an injury, or be driven by the host immune response to the antigenically foreign sperm (Gregory and Cyr 2014). The inflammation of the epididymis results in changes in the local cytokines network (Dubé and Cyr 2013), and in an increased number of inflammatory cells (e.g., macrophages and lymphocytes) within the interstitium along with an increased number of halo cells in the epithelium (Gregory and Cyr 2014). Inflammation of the epididymis has beeen associated with altered levels of interleucins -6, -17, and -23 and tumor necrosis factor-alpha, which interfere with the immunotolerant basal status of the epididymis, serving as chemotactic for the immune cells (Gregory and Cyr 2014). The presence of pathogens in the epididymis also activates Toll receptors, which in turn react by triggering different subsets of inflammatory mediators (mainly the cytokines mentioned above) (Ghosh and Ghosh 2020), prostaglandins and reactive oxigen species (Avellar et al. 2019). Moreover, changes in Toll receptor may affect the indolamin signalling pathway, changing the local conditions of sperm tolerance by changing the T-lymphocytes sub-sets present in the interstitium (Guiton, Henry-Berger, and Drevet 2013). The changes in the

cytokines have also been associated with an altered expression of TJs integrity (Dubé and Cyr 2013), which may be mediated by a decreased availability of claudins for the junctional complexes. The loss of epithelial integrity would favour fluid and cells leakage toward the interstitium, and the formation of anti-sperm antibodies and granuloma.

Inflammation may additionally foster infertility via the TGFβ pathway. A decrease in claudins has been reported to follow an increase in TGFβ isoforms, supporting the idea that the BEB tightness may be affected by various cytokines (Stammler et al. 2013). It is possible that, in a particular inflammatory condition, this loosening of BEB would account for an earlier mechanism allowing dendritic cell migration into the epididymal epithelium to control the immunitary challenge (Stammler et al. 2013).

A compromise of BEB integrity follows the exposure to some environmental toxicants, such as the endocrine disruptors (Avellar et al. 2019). The later often act through the retinoid acid pathway (Novák, Benísek, and Hilscherová 2008). It has been shown that some toxicants are able to bind and activate the nuclear retinoid receptors (Lombó et al. 2019), thereby compromising the retinoic acid signalling pathways in the epididymis. In addition, environmental toxicants also affect androgen secretion in the testis (Cyr, Dufresne, and Gregory 2018), reinforcing the dysruption of the BEB functions. Some endocrine disruptors, like biphenyls, alter the BEB permeability by decreasing the expression of ZO and other TJs proteins (Gregory and Cyr 2014).

CONCLUSION

It is believed that the BEB is the major structure providing protection to spermatozoa after leaving the seminiferous tubules. It provides an immune priviledged space for the sperm cells transiting the epididymis, and actively controls the fluid constitution nurturing the sperm maturation process and its fertilizing ability, by tunning the fluid constitution to the specific needs of the spermatozoa at each region of the epididymis. At the

core of these functions, are different cell types, interconnected by different junctional complexes to collaboratively provide an environment designed to warrant male fertility.

Despite its importance, the BEB is less known that other blood-tissues barriers. This review intent to provide a brief description of the BEB constituents, tacking the cellular interactions and functions, and briefly approaching the barrier regulation. Albeit the regulatory mechanisms are not fully portrayed, this chapter also attempted to discuss the dysregulation of BEB during male infertility, hoping to provide the reader with a concise reference on the blood-epididymal barrier importance to male (in)fertility.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to Mr. Pedro Pinto, for the graphic illustrations in this chapter, Dr Joana Reis for her critical review of the document content and language and Luisa Fialho for her technical support with slides preparation.

REFERENCES

Akbarsha, M. A. Faisal, K. Radha, A. 2016. “The Epididymis: Structure and Function.” In *Mammalian Endocrinology and Male Reproductive Biology*, edited by S. Singh, 132-183. Boca Raton: CRC Press.

Aktuğ, H., K. Özdedeli, B. Altay, İ Cüreklibatir, Ö Yilmaz, S. Albay, K. Ergin, and M. Turgut. 2006. “Postpubertal Testicular/Epididymal Epithelial Thickness Alterations in Unilateral Epididymal/Vasal Obstruction of Prepubertal Rats.” *Arch Androl* 52 (6):417-421. doi: 10.1080/01485010600840723.

Anderson, J. M. 2001. “Molecular structure of tight junctions and their role in epithelial transport.” *News Physiol Sci* 16:126-30. doi: 10.1152/physiologyonline.2001.16.3.126.

Angulo-Urarte, A., T. van der Wal, and S. Huveneers. 2020. "Cell-cell junctions as sensors and transducers of mechanical forces." *Biochim Biophys Acta Biomembr* 1862 (9):183316. doi: 10.1016/j.bbamem.2020.183316.

Astraudo, C., A. Lefèvre, F. Boué, F. Dürr, and C. Finaz. 1995. "In vivo regulation of rat epididymal proteins by retinoids: analysis by two-dimensional electrophoresis." *Arch Androl* 35 (3):247-59. doi: 10.3109/01485019508987877.

Avellar, M. C. W., C. M. Ribeiro, M. R. Dias-da-Silva, and E. J. R. Silva. 2019. "In search of new paradigms for epididymal health and disease: innate immunity, inflammatory mediators, and steroid hormones." *Andrology* 7 (5):690-702. doi: 10.1111/andr.12654.

Bosveld, F., Z. Wang, and Y. Bellaïche. 2018. "Tricellular junctions: a hot corner of epithelial biology." *Curr Opin Cell Biol* 54:80-88. doi: 10.1016/j.ceb.2018.05.002.

Breton, S., A. V. Nair, and M. A. Battistone. 2019. "Epithelial dynamics in the epididymis: role in the maturation, protection, and storage of spermatozoa." *Andrology* 7 (5):631-643. doi: 10.1111/andr.12632.

Breton, S., Y. C. Ruan, Y. J. Park, and B. Kim. 2016. "Regulation of epithelial function, differentiation, and remodeling in the epididymis." *Asian J Androl* 18 (1):3-9. doi: 10.4103/1008-682X.165946.

Britan, A. 2006. *Développement, optimisation et utilisation d'un système cellulaire de l'épithélium épididymaire murin : approches moléculaires.* Doctoral thesis in Génétique et Physiologie Moléculaires, Ecole Doctorale des Sciences de la Vie et de la Santé, Université Blaise Pascal - Clermont-Ferrand I (NT : 2005CLF21652). [*Development, optimization and use of a cell system of the murine epididymal epithelium: molecular approaches*.]

Cyr, D. G. 2011. "Connexins and pannexins: Coordinating cellular communication in the testis and epididymis." *Spermatogenesis* 1 (4):325-338. doi: 10.4161/spmg.1.4.18948.

Cyr, D. G., J. Dufresne, and M. Gregory. 2018. "Cellular junctions in the epididymis, a critical parameter for understanding male reproductive

toxicology." *Reprod Toxicol* 81:207-219. doi: 10.1016/j.reprotox.2018.08.013.

Cyr, D. G., M. Gregory, E. Dubé, J. Dufresne, P. T. Chan, and L. Hermo. 2007. "Orchestration of occludins, claudins, catenins and cadherins as players involved in maintenance of the blood-epididymal barrier in animals and humans." *Asian J Androl* 9 (4):463-75. doi: 10.1111/j.1745-7262.2007.00308.x.

Cyr, Daniel G., Kenneth Finnson, Julie Dufresne, and Mary Gregory. 2002. "Cellular Interactions and the Blood-Epididymal Barrier." In *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*, edited by Bernard Robaire and Barry T. Hinton, 103-118. Boston, MA: Springer US.

Da Silva, N., and C. R. Barton. 2016. "Macrophages and dendritic cells in the post-testicular environment." *Cell Tissue Res* 363 (1):97-104. doi: 10.1007/s00441-015-2270-0.

Da Silva, N., V. Cortez-Retamozo, H. C. Reinecker, M. Wildgruber, E. Hill, D. Brown, F. K. Swirski, M. J. Pittet, and S. Breton. 2011. "A dense network of dendritic cells populates the murine epididymis." *Reproduction* 141 (5):653-63. doi: 10.1530/REP-10-0493.

Dacheux, J. L., and F. Dacheux. 2014. "New insights into epididymal function in relation to sperm maturation." *Reproduction* 147 (2):R27-42. doi: 10.1530/REP-13-0420.

Debbage, Paul, and Gudrun C. Thurner. 2010. "Nanomedicine Faces Barriers." *Pharmaceuticals* 3 (11):3371-3416. doi: 10.3390/ph3113371.

Dube, E., L. Hermo, P. T. Chan, and D. G. Cyr. 2010. "Alterations in the human blood-epididymis barrier in obstructive azoospermia and the development of novel epididymal cell lines from infertile men." *Biol Reprod* 83 (4):584-96. doi: 10.1095/biolreprod.110.084459.

Dubé, E., J. Dufresne, P. T. Chan, L. Hermo, and D. G. Cyr. 2010. "Assessing the role of claudins in maintaining the integrity of epididymal tight junctions using novel human epididymal cell lines." *Biol Reprod* 82 (6):1119-28. doi: 10.1095/biolreprod.109.083196.

Dubé, Évemie, and Daniel G. Cyr. 2013. "The Blood-Epididymis Barrier and Human Male Fertility." In *Biology and Regulation of Blood-Tissue Barriers*, edited by C. Yan Cheng, 218-236. New York, NY: Springer New York.

Dyce, K. M., W. O. Sack, and C. J. G. Wensing. 2010. "The Male Reproductive Organs - The Pelvis and Reproductive Organs of the Dog and Cat." In *Textbook of Veterinary Anatomy*, 466-474. St. Louis, MI, USA: Saunders, Elsevier.

Elfgen, V., A. Mietens, M. Mewe, T. Hau, and R. Middendorff. 2018. "Contractility of the epididymal duct: function, regulation and potential drug effects." *Reproduction* 156 (4):R125–R141. doi: 10.1530/REP-17-0754.

Elzanaty, S. 2007. "Association between age and epididymal and accessory sex gland function and their relation to sperm motility." *Arch Androl* 53 (3):149-56. doi: 10.1080/01485010701225667.

Ezer, Nadine, and Bernard Robaire. 2002. "Androgenic Regulation of the Structure and Functions of the Epididymis." In *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*, edited by Bernard Robaire and Barry T. Hinton, 297-316. Boston, MA: Springer US.

França, Luiz R., Sarah A. Auharek, Rex A. Hess, Jannette M. Dufour, and Barry T. Hinton. 2013. "Blood-Tissue Barriers: Morphofunctional and Immunological Aspects of the Blood-Testis and Blood-Epididymal Barriers." In *Biology and Regulation of Blood-Tissue Barriers*, edited by C. Yan Cheng, 237-259. New York, NY: Springer New York.

Ghosh, Suvendu, and Debosree Ghosh. 2020. "Current perspectives of Male Infertility induced by Immunomodulation due to Reproductive Tract Infections." *Chemical Biology Letters; Vol 7, No 2 (2020)*.

Gregory, M., and D. G. Cyr. 2006. "Identification of multiple claudins in the rat epididymis." *Mol Reprod Dev* 73 (5):580-8. doi: 10.1002/mrd.20467.

Gregory, M., and D. G. Cyr. 2014. "The blood-epididymis barrier and inflammation." *Spermatogenesis* 4 (2):e979619. doi: 10.4161/21565562.2014.979619.

Guiton, R., J. Henry-Berger, and J. R. Drevet. 2013. "The immunobiology of the mammalian epididymis: the black box is now open!" *Basic Clin Androl* 23:8. doi: 10.1186/2051-4190-23-8.

Guiton, R., A. Voisin, J. Henry-Berger, F. Saez, and J. R. Drevet. 2019. "Of vessels and cells: the spatial organization of the epididymal immune system." *Andrology* 7 (5):712-718. doi: 10.1111/andr.12637.

Hall, S. H., S. Yenugu, Y. Radhakrishnan, M. C. Avellar, P. Petrusz, and F. S. French. 2007. "Characterization and functions of beta defensins in the epididymis." *Asian J Androl* 9 (4):453-62. doi: 10.1111/j.1745-7262.2007.00298.x.

Hedger, M. P. 2011. "Immunophysiology and pathology of inflammation in the testis and epididymis." *J Androl* 32 (6):625-40. doi: 10.2164/jandrol.111.012989.

Hedger, Mark P. 2015. "Chapter 19 - The Immunophysiology of Male Reproduction." In *Knobil and Neill's Physiology of Reproduction (Fourth Edition)*, edited by Tony M. Plant and Anthony J. Zeleznik, 805-892. San Diego: Academic Press.

Hess, Rex A., Qing Zhou, and Rong Nie. 2002. "The Role of Estrogens in the Endocrine and Paracrine Regulation of the Efferent Ductules, Epididymis and Vas Deferens." In *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*, edited by Bernard Robaire and Barry T. Hinton, 317-337. Boston, MA: Springer US.

Higashi, T., and H. Chiba. 2020. "Molecular organization, regulation and function of tricellular junctions." *Biochim Biophys Acta Biomembr* 1862 (2):183143. doi: 10.1016/j.bbamem.2019.183143.

Higashi, T., and A. L. Miller. 2017. "Tricellular junctions: how to build junctions at the TRICkiest points of epithelial cells." *Mol Biol Cell* 28 (15):2023-2034. doi: 10.1091/mbc.E16-10-0697.

Isasti-Sanchez, Jone, Fenja Münz-Zeise, and Stefan Luschnig. 2020. "Transient opening of tricellular vertices controls paracellular transport

through the follicle epithelium during Drosophila oogenesis." *bioRxiv*:2020.02.29.971168. doi: 10.1101/2020.02.29.971168.

Jauregui, E. J., D. Mitchell, T. Topping, C. A. Hogarth, and M. D. Griswold. 2018. "Retinoic acid receptor signaling is necessary in steroidogenic cells for normal spermatogenesis and epididymal function." *Development* 145 (13). doi: 10.1242/dev.160465.

Kirchhoff, C. 2018. "Genetische Heterogenität der angeborenen Samenleiteraplasie // Genetic Heterogeneity of Congenital Vas Deferens Aplasia." *Journal für Reproduktionsmedizin und Endokrinologie - Journal of Reproductive Medicine and Endocrinology* 15 (3):136-142.

Krstić, R. V. 1991. "Urogenital Apparatus." In *Human Microscopic Anatomy: An Atlas for Students of Medicine and Biology*, edited by Radivoj V. Krstić, 295-437. Berlin, Heidelberg: Springer Berlin Heidelberg.

Letizia, A., D. He, S. Astigarraga, J. Colombelli, V. Hatini, M. Llimargas, and J. E. Treisman. 2019. "Sidekick Is a Key Component of Tricellular Adherens Junctions that Acts to Resolve Cell Rearrangements." *Dev Cell* 50 (3):313-326.e5. doi: 10.1016/j.devcel.2019.07.007.

Li, Michelle W. M., Dolores D. Mruk, and C. Yan Cheng. 2013. "Gap Junctions and Blood-Tissue Barriers." In *Biology and Regulation of Blood-Tissue Barriers*, edited by C. Yan Cheng, 260-280. New York, NY: Springer New York.

Lombó, M., C. Fernández-Díez, S. González-Rojo, and M. P. Herráez. 2019. "Genetic and epigenetic alterations induced by bisphenol A exposure during different periods of spermatogenesis: from spermatozoa to the progeny." *Sci Rep* 9 (1):18029. doi: 10.1038/s41598-019-54368-8.

Lotti, F., E. Baldi, G. Corona, F. Lombardo, E. Maseroli, S. Degl'Innocenti, L. Bartoli, and M. Maggi. 2018. "Epididymal more than testicular abnormalities are associated with the occurrence of antisperm antibodies as evaluated by the MAR test." *Hum Reprod* 33 (8):1417-1429. doi: 10.1093/humrep/dey235.

Lowe, James S., and Peter G. Anderson. 2015. "Chapter 16 - Male Reproductive System." In *Stevens & Lowe's Human Histology (Fourth Edition) (Fourth Edition)*, edited by James S. Lowe and Peter G. Anderson, 319-336. Philadelphia: Mosby.

Mandon, M., and D. G. Cyr. 2015. "Tricellulin and its role in the epididymal epithelium of the rat." *Biol Reprod* 92 (3):66. doi: 10.1095/biolreprod.114.120824.

Mital, P., B. T. Hinton, and J. M. Dufour. 2011. "The blood-testis and blood-epididymis barriers are more than just their tight junctions." *Biol Reprod* 84 (5):851-8. doi: 10.1095/biolreprod.110.087452.

Noblanc, A., A. Klaassen, and B. Robaire. 2020. "The Exacerbation of Aging and Oxidative Stress in the Epididymis of." *Antioxidants (Basel)* 9 (2). doi: 10.3390/antiox9020151.

Novák, J., M. Benísek, and K. Hilscherová. 2008. "Disruption of retinoid transport, metabolism and signaling by environmental pollutants." *Environ Int* 34 (6):898-913. doi: 10.1016/j.envint.2007.12.024.

Ong, David E., Marcia E. Newcomer, Jean-Jacques Lareyre, and Marie-Claire Orgebin-Crist. 2000. "Epididymal retinoic acid-binding protein." *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymo* 1482 (1):209-217. doi: 10.1016/ S0167-4838(00)00156-4.

Orgebin-Crist, Marie-Claire, Jean-Jacques Lareyre, Kichiya Suzuki, Yoshihiko Araki, Sophie Fouchécourt, Robert J. Matusik, and David E. Ong. 2002. "Retinoids and Epididymal Function." In *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*, edited by Bernard Robaire and Barry T. Hinton, 339-352. Boston, MA: Springer US.

Pal, P. C., M. Manocha, M. M. Kapur, D. N. Rao, R. S. Sharma, and M. Rajalakshmi. 2006. "Obstructive infertility: changes in the histology of different regions of the epididymis and morphology of spermatozoa." *Andrologia* 38 (4):128-36. doi: 10.1111/j.1439-0272.2006.00726.x.

Park, Y. J., M. A. Battistone, B. Kim, and S. Breton. 2017. "Relative contribution of clear cells and principal cells to luminal pH in the

mouse epididymis." *Biol Reprod* 96 (2):366-375. doi: 10.1095/biolreprod.116.144857.

Payan-Carreira, R., and D. Santos. 2020. "*Cadherin-Mediated Cell Adhesion within the Seminiferous Tubules*." In Cadherins: Types, Structure and Functions, edited by J. McWilliam, 95-127. Nova Science Publishers, Inc. ISBN: 978-1-53618-077-0

Pierucci-Alves, F., M. T. Midura-Kiela, S. D. Fleming, B. D. Schultz, and P. R. Kiela. 2018. "Transforming Growth Factor Beta Signaling in Dendritic Cells Is Required for Immunotolerance to Sperm in the Epididymis." *Front Immunol* 9:1882. doi: 10.3389/fimmu.2018.01882.

Rajasekaran, S. A., K. W. Beyenbach, and A. K. Rajasekaran. 2008. "Interactions of tight junctions with membrane channels and transporters." *Biochim Biophys Acta* 1778 (3):757-69. doi: 10.1016/j.bbamem.2007.11.007.

Ribeiro, C. M., E. J. Silva, B. T. Hinton, and M. C. Avellar. 2016. "β-defensins and the epididymis: contrasting influences of prenatal, postnatal, and adult scenarios." *Asian J Androl* 18 (2):323-8. doi: 10.4103/1008-682X.168791.

Robaire, Bernard. 2002. "Aging of the Epididymis." In *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*, edited by Bernard Robaire and Barry T. Hinton, 285-296. Boston, MA: Springer US.

Robaire, Bernard, and Barry T. Hinton. 2015. "Chapter 17 - The Epididymis." In *Knobil and Neill's Physiology of Reproduction (Fourth Edition)*, edited by Tony M. Plant and Anthony J. Zeleznik, 691-771. San Diego: Academic Press.

Sarda, A. K., D. Pandey, S. A. Bhalla, S. Gupta, and N. Khurana. 2011. "Effect of obstruction to sperm egress on the male testis and epididymis." *Internet J Urol* 8 (1):pp. 8.

Serre, V., and B. Robaire. 1999. "Distribution of immune cells in the epididymis of the aging Brown Norway rat is segment-specific and related to the luminal content." *Biol Reprod* 61 (3):705-14. doi: 10.1095/biolreprod61.3.705.

Shum, W. W., N. Da Silva, D. Brown, and S. Breton. 2009. "Regulation of luminal acidification in the male reproductive tract via cell-cell crosstalk." *J Exp Biol* 212 (Pt 11):1753-61. doi: 10.1242/jeb.027284.

Shum, W. W., N. Da Silva, M. McKee, P. J. Smith, D. Brown, and S. Breton. 2008. "Transepithelial projections from basal cells are luminal sensors in pseudostratified epithelia." *Cell* 135 (6):1108-17. doi: 10.1016/j.cell.2008.10.020.

Shum, W. W., Y. C. Ruan, N. Da Silva, and S. Breton. 2011. "Establishment of cell-cell cross talk in the epididymis: control of luminal acidification." *J Androl* 32 (6):576-86. doi: 10.2164/jandrol.111.012971.

Stammler, A., D. Müller, Y. Tabuchi, L. Konrad, and R. Middendorff. 2013. "TGFβs modulate permeability of the blood-epididymis barrier in an in vitro model." *PLoS One* 8 (11):e80611. doi: 10.1371/journal.pone.0080611.

Sullivan, R., C. Légaré, J. Lamontagne-Proulx, S. Breton, and D. Soulet. 2019. "Revisiting structure/functions of the human epididymis." *Andrology* 7 (5):748-757. doi: 10.1111/andr.12633.

Sullivan, Robert, and Clémence Belleannée. 2018. "Cell Biology of the Epididymis." In *Encyclopedia of Reproduction (Second Edition)*, edited by Michael K. Skinner, 286-291. Oxford: Academic Press.

Touré, A. 2019. "Importance of SLC26 Transmembrane Anion Exchangers in Sperm Post-testicular Maturation and Fertilization Potential." *Front Cell Dev Biol* 7:230. doi: 10.3389/fcell.2019.00230.

Trimble, W. S., and S. Grinstein. 2015. "Barriers to the free diffusion of proteins and lipids in the plasma membrane." *J Cell Biol* 208 (3):259-71. doi: 10.1083/jcb.201410071.

Turner, T. T., D. Bomgardner, J. P. Jacobs, and Q. A. Nguyen. 2003. "Association of segmentation of the epididymal interstitium with segmented tubule function in rats and mice." *Reproduction* 125 (6):871-8. doi: 10.1530/rep.0.1250871.

Voisin, A., C. Damon-Soubeyrand, S. Bravard, F. Saez, J. R. Drevet, and R. Guiton. 2020. "Differential expression and localisation of TGF-β

isoforms and receptors in the murine epididymis." *Sci Rep* 10 (1):995. doi: 10.1038/s41598-020-57839-5.

Voisin, A., F. Saez, J. R. Drevet, and R. Guiton. 2019. "The epididymal immune balance: a key to preserving male fertility." *Asian J Androl* 21 (6):531-539. doi: 10.4103/aja.aja_11_19.

Voisin, A., M. Whitfield, C. Damon-Soubeyrand, C. Goubely, J. Henry-Berger, F. Saez, A. Kocer, J. R. Drevet, and R. Guiton. 2018. "Comprehensive overview of murine epididymal mononuclear phagocytes and lymphocytes: Unexpected populations arise." *J Reprod Immunol* 126:11-17. doi: 10.1016/j.jri.2018.01.003.

Wen, Q., E. I. Tang, Y. Gao, T. T. Jesus, D. S. Chu, W. M. Lee, C. K. C. Wong, Y. X. Liu, X. Xiao, B. Silvestrini, and C. Y. Cheng. 2018. "Signaling pathways regulating blood-tissue barriers - Lesson from the testis." *Biochim Biophys Acta Biomembr* 1860 (1):141-153. doi: 10.1016/j.bbamem.2017.04.020.

Witkin, S. S., J. Jeremias, A. M. Bongiovanni, and M. G. Munoz. 1996. "Immune regulation in the male genital tract." *Infect Dis Obstet Gynecol* 4 (3):131-5. doi: 10.1155/S1064744996000294.

Zhang, Y. L., J. S. Zhang, Y. C. Zhou, Y. Zhao, and M. J. Ni. 2011. "Identification of microRNAs and application of RNA interference for gene targeting in vivo in the rat epididymis." *J Androl* 32 (6):587-91. doi: 10.2164/jandrol.111.013060.

Zhou, W., G. N. De Iuliis, M. D. Dun, and B. Nixon. 2018. "Characteristics of the Epididymal Luminal Environment Responsible for Sperm Maturation and Storage." *Front Endocrinol (Lausanne)* 9:59. doi: 10.3389/fendo.2018.00059.