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Review

Are the basal cells of the mammalian epididymis still an enigma?

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Abstract. Basal cells are present in the columnar pseudostratified epithelium covering the epididymis of all mammalian species, which regulates the microenvironment where the functionally incompetent germ cells produced by the testis are matured and stored. Striking novelties have come from investigations on epididymal basal cells in the past 30–40 years. In addition to an earlier hypothesised scavenger role for basal cells, linked to their proven extratubular origin and the expression of macrophage antigens, basal cells have been shown to be involved in cell–cell cross-talk, as well as functioning as luminal sensors to regulate the activity of principal and clear cells. Involvement of basal cells in the regulation of electrolyte and water transport by principal cells was hypothesised. This control is suggested to be mediated by the local formation of prostaglandins. Members of the aquaporin (AQP) and/or aquaglyceroporin family (AQP3, AQP7 and AQP8) are also specifically expressed in the rat epididymal basal cells. Transport of glycerol and glycerylphosphorylcholine from the epithelium of the epididymis to the lumen in relation to sperm maturation may be mediated by AQP. Most probably basal cells collaborate to the building up of the blood–epididymis barrier through cell adhesion molecules, implying an involvement in immune control exerted towards sperm cells, which are foreigners in the environment in which they were produced.

Additional keywords: epithelium, histophysiology.

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About the enigma: general and historical background

The epididymis is the convoluted tubule connecting the efferent ducts to the vas deferens. When uncoiled, the tubule is several meters long (in mammals, ranging from 3 to 80 m). This is the place where the functionally incompetent germ cell produced by the testis is matured and stored. During passage through the epididymal duct (around 1–2 weeks in most species), the spermatozoon undergoes many changes that prepare it to carry out the tasks required of it. The regulation of the epididymal microenvironment and the manifold ways by which it influences sperm maturation have been the subject of research for many years. The epithelium lining the duct is columnar pseudostratified throughout its length. In every mammalian species and tract, the epithelial lining consists mainly of non-ciliated (principal) cells and basal cells, the principal cells being the primary cell type throughout the tubule, comprising approximately 80% of the epithelium and responsible for the majority of the proteins secreted into the lumen. Other cell types are present depending on the species, including apical cells, narrow cells, clear cells and halo cells. Narrow, apical and clear cells intensely express the vacuolar proton-pumping ATPase (V-ATPase) in their apical membrane and are known to account for proton secretion into the lumen, thus participating in its acidification (Breton *et al.* 1996; Pastor-Soler *et al.* 2005; Pietrement *et al.* 2006; Da Silva *et al.* 2007; Kujala *et al.* 2007; Belleannée *et al.* 2010), which is essential for sperm inactivity during their transition into and storage in the epididymis. Principal cells and clear cells are also responsible for the clearance of proteins from the epididymal lumen by endocytosis (Vierula *et al.* 1995).

There are differences in the distribution of the diverse cell types present in the adult epididymis of the mammalian species studied to date. In humans and rodents, some cell types may be present in the epithelium of a specific region (e.g. initial segment; narrow and/or apical cells), others are present in several regions (e.g. caput, corpus and cauda; clear cells) and others are found in all regions (principal, basal cells; Hamilton 1975; Hermo *et al.* 1994; Cornwall *et al.* 2002; Robaire *et al.* 2006). In addition to resident cell types, wandering blood cells can be found scattered in the epithelium. Initially called ‘halo cells’, these cells were successively identified as lymphocytes and monocytes and/or macrophages (Flickinger *et al.* 1997; Serre and Robaire 1999). In other mammalian species, the epithelial lining has a smaller number of cell types. In carnivores, the epithelium is basically comprised of principal and basal cells plus randomly distributed apical cells (Chandler *et al.* 1981; Arrighi *et al.* 1986; Chimming and Vicentini 2001). Similarly, in ungulates, the epithelium is lined by principal,

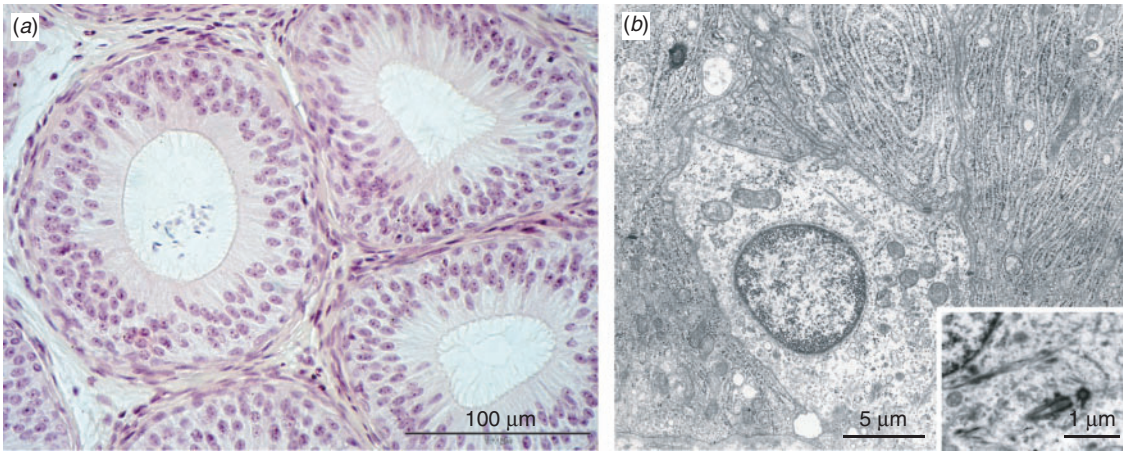

Fig. 1. Morphological aspects of the epididymal basal cells. (*a*) Haematoxylin and eosin-stained corpus of the epididymis from an adult dog. The basal cells are lying on the basal membrane, nested among principal cells. In carnivores, the basal cells are characteristically roundish and almost form a monolayer. (*b*) Ultrastructure of the basal cells in the corpus region of the cat epididymis. The basal cell, resting on the basal membrane, is roundish, has a euchromatic nucleus and pale cytoplasm and a paucity of organelles. In the micrograph, the basal region of adjacent principal cells can also be seen, the cytoplasm of which is crowded with stacks of granular endoplasmic reticulum. Adjacent basal and principal cells are interconnected by cytoplasmic extensions and membrane digitations. The inset shows a solitary cilium in the basal cell of the horse epididymis (reproduced with permission from Elsevier). See text for details.

apical and basal cells (Nicander 1958; Goyal 1985; Goyal and Williams 1991; Arrighi *et al.* 1991, 1993, 2010*b*; Schön and Blottner 2009; Alkafafy *et al.* 2012). Some information also exists for feral mammals, such as the elephant, whose epididymis is comprised mainly of principal and basal cells plus macrophage-resembling halo cells (Holt *et al.* 1980). In studies on the evolution and adaptive implications of epididymal function, the male excurrent duct was also investigated in non-eutherian mammals. In the tammar wallaby, the epididymal epithelium is composed of principal, mitochondria-rich, apical and basal cells, as well as intraepithelial leucocytes (Jones *et al.* 1984). In the echidna, Bedford and Rifkin (1979) evidenced a lesser regional complexity of the epithelium that characterises this duct compared with that in the Theria, together with a much closer similarity to that of non-passerine birds. Nonetheless, the epithelium in this lower mammal is composed of principal, apical, basal and halo cells (Djakiew and Jones 1981).

In fact, even if the general organisation of the epithelium lining the mammalian epididymis is almost the same across species, differences and peculiarities are frequent, mirroring species-specific functional modulations. This is true also for regional modulations of the duct, which vary considerably among species in such a way that results from one species cannot be assumed to infer regional functions in another.

Basal cells are present in the epididymal epithelium of all species. They are located on the basal lamina, as in several other pseudostratified epithelia, and they are reputed never to reach the lumen. Such a type of simple epithelium, in which all the cells rest on the basal lamina and the nuclei of the epithelial cells are seen at different levels, thus creating the illusion of cellular stratification, can be found in the ciliated pseudostratified columnar epithelia lining the upper respiratory tract or the uterine tube in most mammals, and in the non-ciliated pseudostratified columnar epithelium lining the epididymis and vas deferens.

More than 35 years ago, the eminent researcher David W. Hamilton stated, in a renowned review on epididymal structure and function, that ‘epididymal basal cells are an enigma’ (Hamilton 1975). At that time, basal cells were supposed to sustain a stem function in the renewal of the epithelium (Hamilton 1975; Ramos and Dym 1977; Jones *et al.* 1979). This hypothesis, together with the opposing view that principal cells may give rise to basal cells (Sun and Flickinger 1982), was contradicted by the demonstration by the [^{3}H]-thymidine uptake of low mitotic activity in the rat epididymis (Sujarit and Jones 1991). Others also suggested a mere mechanical role for the basal cells in providing stability of the epithelium because of the presence of cytoplasmic filament bundles and membrane interdigitations (Ramos and Dym 1977; Tingari 1989). Much of the morphological research in mammals was performed in the 1980s and 1990s but, in recent years, the epididymal basal cell has continued to attract researchers’ attention.

Morphological aspects in mammalian species

The morphology of epididymal basal cells shows similarities across mammalian species. The cells are oval or round in shape, with large, round and euchromatic nuclei and a pale cytoplasm, and rest on the basal lamina. In most micrographs, the epididymal basal cells do not reach the lumen (Fig. 1*a*). In the murine epididymis, the epididymal basal cells extend along the basement membrane so as to cover a large part of the circumference of the epididymal duct. In some species and epididymal tracts, basal cells are so numerous to form a monolayer at the base of the epithelium, being nestled beneath principal cells. There is general agreement that basal cells are present in greater number in the corpus of the epididymis and less numerous towards the cauda (Goyal and Dhingra 1975; Arrighi *et al.* 1991; Schön and Blottner 2009; Arrighi 2013).

In contrast with the predominant opinion, it has been definitively shown in the murine epididymis that basal cells possess slender cytoplasmic extensions that cross the apical barrier of the tight junction formed by the principal cells joining with one another (creating, in turn, the blood–epididymis barrier) and, thus, reach the epithelial lumen (Shum *et al.* 2008). Crossing the blood–epididymis barrier without destroying its integrity may be achieved by creating new tight junctions between basal cells and adjacent epithelial cells in a dynamic and temporary way. Regional differences have been noted with regard to the number of basal cells reaching the luminal border, strongly increasing in the distal corpus and proximal cauda (Shum *et al.* 2008). This pattern may indicate that the capacity of basal cells to reach the lumen is regulated locally in different regions of the epididymis. Other mammalian species most probably share the morphology of the basal cells demonstrated in mice.

Ultrastructure

As seen by transmission electron microscopy (TEM) (Fig. 1*b*), basal cells appear poorly differentiated. The cellular outline is characterised by cytoplasmic extensions and membrane infoldings that connect the basal cells to the adjacent epithelial cells. Junctional devices are also present that join the lateral cell membrane of the basal cells to adjacent epithelial cells. In some sections, it is possible to observe that, between the aforementioned junctions, the opposing cell membranes are separated from each other by a tiny space. The most basal area of the cell, which faces the basal membrane, is predominantly straight and connected to the basal lamina by hemidesmosomes. Micropinocytotic vesicles are also frequently present.

The cytoplasm appears to contain few organelles. Dispersed profiles of endoplasmic reticula, a few mitochondria and a small Golgi apparatus are present in the cytoplasm, without fixed localisation. Cytoplasmic lipid droplets are a common observation in large domestic mammals, such as cattle (Goyal 1985; Sinowatz 1981), camel (Tingari 1989), equidae (Arrighi *et al.* 1991, 1993), together with residual bodies that have a heterogeneous aspect. The finding of lipid droplets and residual bodies was considered to be indicative of degradation of exogenous material, and hence phagocytic activity, as suggested in the epididymis of the hamster as early as 1973 (Suzuki and Glover 1973).

Early ultrastructural studies highlighted the presence of microfilament bundles in the cytoplasm of basal cells in the rat (Hamilton 1975), monkey (Ramos and Dym 1977), cat (Arrighi *et al.* 1986), equidae (Arrighi *et al.* 1991, 1993), camel (Tingari 1989). On this basis, most authors hypothesised that the basal cells ensure the stability of the epithelium, together with membrane infoldings. Immunohistochemical evidence has demonstrated the expression or coexpression of different cytokeratins, vimentin and desmin in the epididymis in humans (Kasper and Stosiek 1989; Dinges *et al.*1991; Palacios *et al.* 1993). These studies evidenced a cell-specific pattern of expression. In the dog epididymis, basal cells in the caput and corpus regions contain low molecular weight cytokeratins, whereas those of the corpus region also contain high molecular weight cytokeratins (Wakui *et al.* 1994); the authors could not give a satisfactory explanation for these observations.

In addition, microtubule-associated protein (MAP) 1B is expressed in basal cells of the initial segment and caput epididymis of the rat, but not human (Queiróz *et al.* 2006). MAP1B is a marker of the cytoskeleton and is expressed mainly in neurons of the developing nervous system (Queiróz *et al.* 2006). An augmented number of MAP1B-positive elements was observed in the proximal regions of the epididymis with increasing age (Queiróz *et al.* 2006). The authors speculated that some of the MAP1B-positive basal cells observed in the proximal regions of the rat epididymis may be responsible for some kind of neuroendocrine regulation.

Theory regarding the origin of basal cells

During male embryogenesis, the regions from the caput epididymis to the vas deferens differentiate from the mesonephric duct in a cranial-to-caudal manner, in parallel with their exposure to testicular androgens.

At birth, the epididymis is made mainly of mesenchymal tissue; thereafter, it goes through considerable remodelling until puberty, including duct elongation and convolution. Communication between the epithelium and mesenchyme is a two-way street. Interplay between the stromal and epithelial compartments of the developing reproductive tract is essential for normal epididymal development (Atanassova *et al.* 2005; Archambeault *et al.* 2009).

A series of studies has shown that the epididymal basal cells appear during postnatal development of the organ. According to Seiler *et al.* (1998), the cells appear at 1–2 weeks in mice, at 3–4 weeks in the rat and at 6 weeks in rabbits, whereas in humans basal cells are not present in the epithelium until puberty. The few data on domestic animal species confirm postnatal appearance of basal cells at 4–6 weeks in rams (Nilnophakoon 1978), 10 weeks in goats (Yao and Eaton 1954) and after 12 weeks in cats (Arrighi and Domeneghini 1993). In mice, it was argued that the differentiation of the epididymal epithelium occurring before puberty follows an ascending pattern, which means that basal cells appear in the cauda earlier than in the corpus epididymis and in the corpus earlier than in the caput epididymis (Seiler *et al.* 1998). Moreover, basal cells still appeared in the ligated postpubertal epididymis (Seiler *et al.* 1999); thus, it was suggested that exocrine secretions from testicular origin reaching the epididymis at puberty via the canalicular route did not significantly influence basal cell presence, although they may modulate the expression of macrophage antigens by the basal cells.

Holschbach and Cooper (2002) provided significant evidence that basal cells may arise from extratubular sources in the murine model because a diminished number of mitotic figures in the epithelium was noted as the number of basal cells increased. Moreover, bromodeoxyuridine labelling indicated the probable arrival of basal cells from circulating monocytes coming from the bone marrow.

On the basis of nuclear staining for *p63* in almost the entire basal cell population in the human epididymis (Hayashi *et al.* 2004), it was also demonstrated that the *p63* transcription factor plays essential roles during the differentiation and maintenance of basal cells. The *p63* gene (*Trp63*) encodes two major classes

of protein with different transactivation properties, namely TAp63 and ΔNp63, transcribed from two independent promoters. ΔNp63 is the major form expressed in squamous epithelial cells, including epididymal basal cells, in which it is hypothesised to regulate differentiation (Hayashi *et al.* 2004). The hypothesis that *p63* is essential for basal cell differentiation in the epididymis and that basal cells are not required for the differentiation of principal, narrow and clear cells was further investigated in the developing epididymis using *p63*-knockout mice. The single-layered epithelia of p63-deficient epididymides lacked basal cells, but the expression of molecular markers for principal, narrow and clear cells was intact (Murashima *et al.* 2011).

Atanassova *et al.* (2005) demonstrated that transient neonatal treatment of rats with high doses of the potent oestrogen diethylstilboestrol results in a considerable variety of abnormal changes, including anomalous twisting of the vas deferens, abnormalities of the epithelium and stromal cell anomalies, such as an increase in the thickness of the periductal fibroblast layer and diminished epithelial androgen receptors (AR). One of the epithelial abnormalities was delayed basal cell development, thus evidencing a role for androgens and oestrogens in basal cell development and suggesting that this may be crucial in determining normal epithelial and stromal development of the cauda and vas deferens (Atanassova *et al.* 2005). Moreover, *in vitro* experiments in mice suggested that *p63* is a candidate target of androgen receptor (AR) signaling (Murashima *et al.* 2011).

The ARs are expressed along the human (Ungefroren *et al.* 1997), goat (Goyal *et al.* 1997), stallion (Parlevliet *et al.* 2006), mouse (Zhou *et al.* 2002) and rat (Zaya *et al.* 2012) epididymis in both principal and basal cells.

It is also known that oestrogens play key roles in the development and maintenance of male reproductive function and fertility. In addition to regulating ion transport and water reabsorption in the efferent ducts and epididymis, the oestrogen receptor ERα is also being discovered to regulate the expression of other associated genes (Hess *et al.* 2011). In the male, some genes, such as aquaporin 9 *Aqp9* and the sodium/hydrogen exchanger *Slc9a3*, contain both androgen and estrogen response elements and are doubly regulated by these hormones (Joseph *et al.* 2011). Similar to AR, the oestrogen receptor ERβ is widely expressed throughout the male reproductive tract (Nie *et al.* 2002). Interestingly, in the initial region of the caput epididymis of cats (Nie *et al.* 2002) and in macaques (Saunders *et al.* 2001), strong ERα immunostaining was seen only in basal cells. Neither group provided a satisfactory explanation for these observations.

Scavenger role of basal cells and the macrophage theory

During the 1990s, information was collected that, together, was indicative of a scavenger role for basal cells. That basal cells acquire disintegration products from nearby principal cells, which are in contact with the lumen, and convert them into lipofuscin material through lysosomal activity was determined by concurrent research work from several laboratories in different mammalian species.

Investigations in seasonal and senescent mammals, natural and/or experimental conditions

To the best of the author's knowledge, the first evidence of a scavenger role for basal cells came from studies of the morphological involutions taking place in mammals with a seasonal breeding pattern, such as the mole (Suzuki and Racey 1976). During sexual regression, the epithelial cells were heavily laden with lipofuscin pigment granules and the basal cells were hypothesised to act as scavenger of the epithelium. Similar observations were made later in hamsters subjected experimentally to a short photoperiod (Calvo *et al.* 1997), as well as in the viscacha during its annual reproductive cycle (Aguilera-Merlo *et al.* 2005).

Inferences derived from studies in senescent males

Observations on the morphological changes in aging rats (Serre and Robaire 1998) indicated that, with increasing age, there is a decrease in the number of principal and basal cells in all segments of the epididymis, accompanied by a marked increase in the number of halo cells, located in the basal region of the epithelium and filled with lysosomes. The hypothesis that basal cells may have the ability to become lipofuscin-rich cells after taking up indigested residues from the cytoplasm of principal cells had also been formulated in the case of azoospermia in *Equus asinus* accompanied by massive involution of the epididymal epithelium (Arrighi *et al.* 1983), and was hypothesised as a normal activity of the epithelial basal cells in Equidae (Arrighi *et al.* 1991, 1993). In fact, similar effects were noted in castrated golden hamsters (Suzuki and Glover 1973).

Some deduction came from simulation experiments, aimed at understanding the potential *in vivo* spermatotoxic effect of different substances, like aflatoxin or chromium, in men who are professionally or environmentally exposed to them. In response to administration of subchronic doses of aflatoxin B1, Agnes and Akbarsha (2001) observed pale vacuolated epithelial cells (PVECs) in the mouse epididymal epithelium. The authors interpreted the PVECs to be basal cells that had presumably enveloped degenerated and disintegrated principal cells. Similar PVECs had been observed after ligation of the epididymal duct in mice (Abe *et al.*1982) and in the epididymis of androgen-deprived goats (Goyal *et al.* 1994). Together, these results suggested an endocytotic capability of epididymal basal cells and successive digestion by lysosomal activity, following uptake and absorption, and it was concluded that the PVEC may develop from the basal cell as a protective device against the autoimmune reaction to spermatozoa (Agnes and Akbarsha 2001). Subsequent research in the bonnet macaque (*Macaca radiata* Geoffroy), aimed at studying the spermatotoxic effect of hexavalent chromium (CrVI) on epididymal epithelial cells and intraepithelial macrophages (Aruldhas *et al.* 2006), showed an increase in basal cells and intraepithelial macrophages and the amount of lipofuscin in both cell types. According to the authors, the dead spermatozoa resulting from CrVI exposure were phagocytosed and processed into lipofuscin material by principal cells. In turn, basal cells and intraepithelial macrophages may acquire this material and process it further.

Moreover, lipofuscin-rich basal cells and intraepithelial macrophages were supposed to leave the epithelium, followed by replacement by new basal cells and intraepithelial macrophages.

However, it should be noted that sperm disposal after vasectomy was put in relation to species-specific properties of non-ejaculated spermatozoa. Jean *et al.* (1979) reported that differences exist in human compared with rat spermatozoa in terms of size, resistance to decondensing agents, content and localisation of sulfhydryl groups. These species specificities can explain why vasectomised rats regularly develop large granulomas, whereas this is not prominent in vasectomised men. In the rabbit, the duct of which is relatively distensible, 6 months after vasectomy all the spermatozoa estimated to have been produced by normal testes in that time had accumulated in the epididymis (Moore and Bedford 1978).

In this, as in other items relating to the anatomy and physiology of the reproductive organs, a large range of behaviours exists among mammalian species.

Macrophage antigen expression in basal cells

In agreement with the aforementioned findings, antigenic and ultrastructural similarities to tissue-fixed macrophages were depicted (Yeung *et al.* 1994), as well as the expression of macrophage antigens, providing immune defence against sperm antigens (Seiler *et al.* 1998, 1999). Accordingly, it was demonstrated that basal cells are a migratory cell population originating in the peritubular tissue (Seiler *et al.* 1998; Holschbach and Cooper 2002). Moreover, observations on the relationship between sperm number and condition versus the number of basal cells and their expression of macrophage antigen confirmed the role of basal cells in sperm degradation in the murine epididymis (Seiler *et al.* 2000). Seiler *et al.* (2000) elegantly confirmed the supposition that the epididymis reacts to an increased number of degenerating spermatozoa by recruiting a larger number of basal cell, by increasing their expression of macrophage antigens or both. Three different experiments were performed in mice, aimed at stressing the epithelium: (1) ligating the genital tract; (2) applying a local thermal stress; and (3) withdrawing testicular androgens in such a way as to increase the number of spermatozoa present in a given region of the tubule and to induce their degeneration (Seiler *et al.* 2000). The cellular composition of the epididymal epithelium was modified in all the experiments with regard to the number of basal cells and their antigen expression. The results were consistent with the view that epididymal basal cells react to the presence of luminal sperm autoantigens by increasing their number and antigen expression.

It should be mentioned that a recent, beautiful study by Da Silva *et al.* (2011) reported the occurrence of a dense network of dendritic cells located at the base of the murine epididymal epithelium, never described before. Epididymal dendritic cells (eDCs) express numerous leucocyte markers. These cells are intimately connected with the epithelium and send long projections between epithelial cells towards the lumen. Da Silva *et al.* (2011) hypothesised that eDCs should be considered as specialised antigen-presenting cells, strategically positioned, together with macrophages, to regulate the complex balance between immune tolerance and activation, which is necessary to sustain fertility in the male. Unfortunately, the authors did not discuss the eventual relationship of eDCs with epididymal basal cells.

The cell surface and the role of basal cells in the blood–epididymis barrier

Cell adhesion molecules, tight junctional proteins

It is well known that the immunological status of the spermatozoa is peculiar, because the post-meiotic germ cells start expressing novel proteins at puberty, long after the setting up of systemic immune tolerance. They are, therefore, foreigners in the environment in which they were produced. The probable existence of a blood–testis barrier was inferred in the early 1900s with the demonstration that certain dyes introduced into the bloodstream were excluded from the testicular seminiferous tubules (Setchell and Waites 1975). The existence of a blood–epididymis barrier was hypothesised much later by convergence of several studies, until morphological evidence was provided (Hoffer and Hinton 1984; Hinton 1985). Generally described as originating from the presence of tight junctions between epithelial cells in the epididymis (Hinton and Palladino 1995; Levy and Robaire 1999; Cyr *et al.* 2007), it is now recognised that the (physiological) blood–epididymis barrier is actually a much more complex structure. According to Mital *et al.* (2011), the tight junctions form the anatomical (i.e. physical) barrier limiting the passage of molecules and cells from or to the lumen. The physiological barrier includes transporters that regulate the movement of substances in or out of the lumen. In turn, the immunological barrier limits contact by the immune system and sequesters the majority of autoantigenic germ cells. None of these three components creates a complete functional barrier on its own; it is the interaction between all three components that creates a maximally competent barrier.

With regard to the anatomical counterpart of the blood–epididymis barrier, the molecular components have been shown to be rather complicated. Dubé *et al.* (2007) demonstrated that the composition of this barrier in the human varies along the duct. Epithelial cell–cell interactions are mediated by adhering and tight junctions between adjacent principal cells. The proteins constituting the epididymal *zonulae adherentes* and tight junctions appear to be conserved between the rodent animal models and human. The cellular contacts in the epididymis involve complex connections between multimember families of proteins and are complicated by the additional influence of testicular factors that regulate the expression of epididymal genes and cellular targeting of proteins. According to Dubé and Cyr (2012), changes in the function of cellular junctions in the human epididymis are clearly associated with male infertility.

The cell adhesion molecules are proteins mostly located on the cell surface of the epididymal principal cells. Of the many epithelial cell contacts, the *zonulae occludentes* of the epididymis are probably the most highly developed. However, α-catenin and claudin 1 (CLDN1) were detected between principal and basal cells in rodents, CLDN1, CLDN3 and

CLDN4 were found between basal and principal cells in all three segments of the human epididymis and CLDN8 was found between basal and principal cells in the corpus region (Cyr *et al.* 2007). In addition, a recent immunohistochemical analysis of human epididymal tissue revealed a specific signal for dysadherin confined mainly to basal cells (Gabrielli *et al.* 2011), but the authors did not provide suggestions as to the functional implications of this result.

Cell–cell cross-talk in the epididymal epithelium

Gap junctional proteins

Different studies into the morphology and localisation of gap junctions in the epididymis have shown that molecules forming the connexins, specifically connexin (Cx) 43, are present between clear and basal cells and between myoid cells, particularly in the distal regions of the rat epididymis (Cyr 2011). The observation that the connexins exchanged between principal and basal cells differed from those exchanged between adjacent principal cells, or between principal and clear cells, led to the speculation that the presence of basal cells may regulate intercellular communication in the epididymis and that basal cells are fundamental for the coordinated functions of the epididymal epithelium. (Cyr *et al.* 1996). The observations that Cx43 also localises between principal and basal cells in the water buffalo, stallion and human suggests that the intercellular communication between basal and principal cell has been conserved among species, in an important fashion for epididymal function (Cyr 2011). Other members of the gap junction protein superfamily, so-called pannexins, are localised in the basal region of the epididymal epithelium. Specifically, pannexin (Panx) 1 appears to be expressed by principal and basal cells. There is evidence that basal cells can reach the lumen of the epididymis and it has been proposed that they may be involved in monitoring the luminal environment and modulating the functions of adjacent cells (Shum *et al.* 2008). Moreover, studies have shown that basal cells release paracrine factors, such as prostaglandin E_2, into the extracellular space, with consequent increase in intracellular cAMP and activation of plasma membrane channels (Leung *et al.* 2004), which may thereby modulate secretions from principal cells. Accordingly, the presence of Panx1 in basal cells of the epididymis may regulate principal cell functions and the secretion of ATP into the basal extracellular space (Dufresne *et al.* 2003; Cyr 2011).

Basal cells as luminal sensors regulating functions of principal cells

In recent years a new and interesting role has been attributed to epididymal basal cells, which may monitor the luminal moiety and control epithelial function by a peculiar cross-talk with other epithelial cells. According to Shum *et al.* (2008), this may be a morphophysiological pattern shared by all basal cells located in the so-called pseudostratified epithelia.

Using high-resolution three-dimensional confocal imaging, Shum *et al.* (2008) demonstrated that basal cells, double-stained for cytochrome *c* oxidase I, a marker of basal cells, and Cldn1 (another marker of basal cells), may reach the lumen through long and slender cytoplasmic projections penetrating between other epithelial cells. In light of these and previous findings, Shum *et al.* (2008) concluded that basal cells are able to cross the blood–epididymis barrier to monitor the luminal milieu.

Angiotensin II is one of the molecules that may be detected in the lumen through specific receptors expressed in basal cells, namely angiotensin AT_2 receptors. Angiotensin II activates proton secretion by clear cells as a result of cross-talk between basal cells and adjacent clear cells (Shum *et al.* 2011). Moreover, other molecules are expressed by basal cells, including the Yf subunit of the glutathione S-transferase (Veri *et al.* 1993) and the Cu^{2+}, Zn^{2+}-superoxide dismutase (Nonogaki *et al.* 1992), which are involved in protection against reactive oxygen species. A further role of basal cells in preventing electrophilic attack can thus be hypothesised.

Monitoring the intercellular space through solitary cilia

A fortuitous finding from our laboratory during ultrastructural studies on the epididymis in Equidae was related to the occurrence, mostly at the level of the corpus region, of a primary cilium emerging from the basal cell surface and insinuating into intercellular spaces (Fig. 1*b*, inset; Arrighi 2013). There is evidence of an analogous occurrence in the cat epididymis (Arrighi *et al.* 1986); thus, the finding is possibly not unique to the Equidae (Arrighi 2013). Although serendipitous in nature, the discovery of primary cilia in epididymal basal cells is a considerable finding that may contribute to our understanding the functional roles most recently attributed to this cell type. In fact, a monitoring role may be ascribed to the immotile cilia, which could act as chemical receptor for extracellular liquids and luminal fluid in which the maturing spermatozoa are bathed, thus modulating the correct epithelial physiology by cell–cell cross-talk involving the entire epithelium.

Regulation of principal cells through prostaglandins

Evidence was provided that basal cells may control the electrolyte and water transport exerted by principal cells (Leung *et al.* 2004). This regulatory process may occur through the local formation of prostaglandins, a process involving two proteins that, in the epididymal epithelium, are exclusively expressed by basal cells. These proteins are the transient receptor potential (Trp) proteins, functioning as transmembrane pathways for Ca^{2+} entry, and cyclo-oxygenase (COX)-1, a key enzyme for the synthesis of prostaglandins. In the rat epididymis, *COX-1* and *COX-2* mRNA have been detected, however the two isozymes have been differently immunolocalised: COX-1 was exclusively present in basal cells, whereas COX-2 was present in principal cells (Leung *et al.* 2004), increasingly from the epididymal initial segment towards the vas deferens. On this basis, Leung *et al.* (2004) suggested that the basal cells may control the electrolyte transport exerted by the principal cells. In addition, differential immunolocalisation of Trps was demonstrated in different cell types (Leung *et al.* 2004). Specifically, Trp1 and Trp3 were exclusively localised in the basal cells of the cauda epididymis, whereas Trp6 was found in principal cells in the same region. Laser microdissection established that the basal cells are the source of Trp3 (Leung *et al.* 2004).

The demonstration that TRPC3 (transient receptor potential cation channel, subfamily C, member 3) and COX-1 are uniquely expressed by basal cells provided evidence that the basal cells are essential for the integrated functions of the epididymal epithelium. Interactions between basal and principal cells were demonstrated shortly later in short-term coculture (Cheung *et al.* 2005). That study proved, for the first time, that basal cells are able to regulate principal cell electrolyte transport by releasing paracrine factors, with a mechanism similar to that exerted by the endothelial cells over the tone of the smooth muscle of blood vessels. Regulation of cell Ca^{2+} and biosynthesis of prostaglandins appear to be the common pathway in both epididymal basal and vascular endothelial cells. This cell–cell interaction is pivotal for the establishment of a correct epididymal environment for sperm maturation.

AQP-mediated water and solute trafficking

Consistent with the significant water movements taking place throughout the duct, there has been considerable attention recently as to the presence of the AQPs in the epididymis. The AQPs are a class of small, hydrophobic, integral membrane proteins that facilitate rapid passive movement of water. Studies have been performed mainly in rodents (Pastor-Soler *et al.* 2001, 2002, 2010; Badran and Hermo 2002; Hermo *et al.* 2004, 2008; Oliveira *et al.* 2005; Da Silva *et al.* 2006*a*, 2006*b*; Arrighi *et al.* 2010*a*; Hermo and Smith 2011), primates (Fisher *et al.* 1998), sporadically in carnivores (Domeniconi *et al.* 2007, 2008; Arrighi *et al.* 2010*c*; Arrighi and Aralla 2013) and very recently in the equine (Klein *et al.* 2013) and bats (Oliveira *et al.* 2013). Molecules, such as androgens, oestrogens and lumicrine factors, modulate the expression of genes involved in aquaporin-mediated fluid absorption in the efferent ductules and the epididymis.

In the epididymal epithelium, AQP1, AQP2, AQP3, AQP5, AQP7 and AQP9 are the most represented AQPs in the species studied to date, with AQP9 being considered the quintessential AQP of the epididymis. AQP1, largely expressed in the non-ciliated cells of the ductuli efferentes from every species studied to date, is absent from the epididymal epithelium. However, AQP1 is expressed on the endothelium of blood vessels and, limited to the rodent (Badran and Hermo 2002; Oliveira *et al.* 2005) and ovine species (Aralla *et al.* 2010), in the myoid cells surrounding the duct, where AQP7 is also expressed in newborn ovine (Aralla *et al.* 2010). AQP5 is expressed in the rat corpus and caudal regions (Hermo and Smith 2011); AQP9 and AQP11 are present on the microvilli in principal cells and AQP7 localises to lateral and/or basal plasma membranes according to the epididymal region (Domeniconi *et al.* 2008); finally, AQP7 and AQP11 are expressed by some, but not all, basal cells (Hermo *et al.* 2008; Hermo and Smith 2011). In the adult rat AQP3 exclusively delineates basal cells (Hermo *et al.* 2004). The reaction clearly defined the boundaries of basal cells, at times also staining their thin lateral processes, occasionally extending towards the lumen. Hermo *et al.* (2004) reported that the AQP3 immunoreaction is shared by basal cells of the epithelium lining the trachea. After orchidectomy, AQP3 expression vanished in rat basal cells, whereas after efferent duct ligation it diminished markedly (Hermo *et al.* 2004). The reaction was not fully re-established after administration of high levels of testosterone to orchidectomised animals, therefore the authors hypothesised that AQP3 expression in basal cells is regulated, in part, by testosterone, in addition to a luminal factor originating from the testis. The finding of AQP3 and AQP7 in epididymal basal cells was interpreted as being part of the intricate cooperative removal of water and/or small uncharged molecules from the epididymal lumen throughout the epididymis, and especially within the distal cauda, likely involving the different cell types (Hermo *et al.* 2008). Because AQP3 and AQP7 are aquaglyceroporins, permeable to water plus glycerol, their presence in basal cells, together with AQP9 expression in principal cells, may sustain the effective transport of glycerol and glycerylphosphorylcholine from the epithelium to the lumen, where these molecules are known to be pivotal in the process of sperm maturation.

Elkjaer *et al.* (2001) have also demonstrated AQP8 expression in epididymal basal cells, as well as basal cells in the conductive airways. In the airways, it was suggested that AQP8 may be involved in the differentiation of epithelial cells, during maturation or after recovery from injury to the airways (Elkjaer *et al.* 2001). AQP11 has also been immunolocalised in the rat epididymis, with discrete labelling of rare basal cells in the corpus and cauda (Hermo *et al.* 2008).

The redundancy of AQPs expressed within the epididymis may be, in part, a means to ensure that failure of any AQP molecule will not impair the epithelium's physiology, specifically the ability to transport water in and out of lumen and to maintain ionic fluid equilibrium for sperm transport, maturation, and storage. In fact, experiments performed in AQP1-, AQP7- or AQP9-knockout mice demonstrated little consequence on fertility in homozygotes (Hermo and Smith 2011).

A modern functional view: is the enigma solved?

Striking novelties have come from investigations on basal cells of the epididymis in the past 30–40 years, which have been outlined in this review.

First, important findings have revealed the true shape of basal cells, which is not simply roundish or hemispherical, as was thought in the past. In fact, basal cells have been shown to possess thin processes that extend not only along the basement membrane in such a way as to cover a large proportion of the circumference of the epididymal tubule and intermingle with adjacent cells, but also steer in the direction of the lumen. This morphology, together with the presence of a solitary cilium extending from the cell surface into the intercellular spaces, is indicative of a monitoring of the luminal microenvironment and consequent cell–cell cross-talk, possibly regulating the functions of the principal cells. Proteins of the gap junction superfamily, such as connexins and pannexins, are also expressed in the basal cells, permitting us to hypothesise complex interactions between the basal and adjacent cells.

Further roles of basal cells in the regulation of electrolyte and water transport exerted by principal cells have also been hypothesised. This process is suggested to be mediated by the local formation of prostaglandins. Members of the AQP and/or aquaglyceroporin family, such as AQP3, AQP7 and AQP8, are

specifically expressed in the epididymal basal cells of some mammals.

The likely collaboration of basal cells in the building up of the blood–epididymis barrier implies an involvement in immunity control exerted towards sperm cells, which are foreigners in the environment in which they are produced. A further, correlated involvement of the basal cells in the immune defence against sperm antigens can be deduced from the proven extratubular origin of the basal cells and the expression of macrophage antigens, which led to the demonstration of their scavenger role, probably exerted towards overproduced spermatozoa (i.e. in animal species having seasonal reproduction) or those damaged by the spermatotoxic effect of different substances.

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