Blackwell Publishing

Luminal acidification in the epididymis
DOI: 10.1111/j.1745-7262.2007.00299.x

AJA
www.asiaandro.com

·*Review*·

Regulation of vacuolar proton pumping ATPase-dependent luminal acidification in the epididymis

Nicolas Da Silva[1,2], Winnie W. C. Shum[1,2], Sylvie Breton[1,2]

[1]*Program in Membrane Biology/Nephrology Division, Massachusetts General Hospital, Boston, MA 02114, USA*
[2]*Harvard Medical School, Boston, MA 02215, USA*

Abstract

Luminal acidification in the epididymis is an important process for the regulation of male fertility. Low pH and low bicarbonate concentration are among key factors that keep spermatozoa in a dormant state while they mature and are stored in this organ. Although significant bicarbonate reabsorption is achieved by principal cells in the proximal regions of the epididymis, clear and narrow cells are specialized for net proton secretion. Clear cells express very high levels of the vacuolar proton pumping ATPase (V-ATPase) in their apical membrane and are responsible for the bulk of proton secretion. In the present paper, selected aspects of V-ATPase regulation in clear cells are described and potential pathologies associated with mutations of some of the V-ATPase subunits are discussed. *(Asian J Androl 2007 July; 9: 476–482)*

Keywords: proton pump; H^+-ATPase; clear cells; bicarbonate resorption

1 Introduction

The establishment of male fertility depends on the production of a large number of spermatozoa by the testis, followed by several maturation steps, which occur in the male excurrent duct. For example, sperm acquire their capacity to become motile while they travel through the epididymis, but they are kept in a quiescent state during this maturation process [1–7]. The establishment and maintenance of a low luminal pH and bicarbonate concentration in the lumen of the epididymis [8, 9] are among key factors that keep spermatozoa dormant [1, 10]. It is particularly crucial that the bicarbonate concentration be maintained low during their storage period because spermatozoa express an adenylyl cyclase that is directly activated by bicarbonate [11–13]. Capacitation of spermatozoa occurs after mixing with the prostatic and seminal vesicle fluids and during transit through the female reproductive tract. This complex process is accompanied by an influx of bicarbonate, which is abundant in prostatic fluid, leading to a rise in intracellular cAMP and subsequent phosphorylation of several proteins by protein kinase A (PKA) [14, 15]. This bicarbonate-induced cAMP elevation also leads to the inhibition of epithelial Na^+ channel (ENaC), which is located in the sperm membrane. ENaC inhibition contributes to the hyperpolarization that accompanies capacitation [16]. In addition, the calcium channel, CatSper1, located in the sperm membrane and which also participates in capacitation, is strongly activated by alkaline pH [17]. Low luminal pH in the epididymis, therefore, prevents the activation of CatSper1 and capacitation in the epididymis.

The establishment of low pH and a low bicarbonate concentration in the lumen of the epididymis has been known for a number of years, thanks to the pioneering work of several groups [8, 9, 18]. However, the mechanisms responsible for transepithelial acid/base transport

Correspondence to: Dr Sylvie Breton, Massachusetts General Hospital, Simches Research Center, Program in Membrane Biology, 185 Cambridge Street, CPZN 8204, Boston, MA 02114, USA.
Tel: +1-617-726-5785 Fax: +1-617-643-3182
E-mail: sbreton@receptor.mgh.harvard.edu

in the epididymis have been described only recently (reviewed in [19]). Significant sodium-dependent bicarbonate reabsorption occurs in the epididymis [8, 9]. This organ is embryologically related to the kidney with which it shares several acid/base transporters that have been implicated in renal bicarbonate reabsorption, including the apical sodium hydrogen exchanger, NHE3, the basolateral sodium bicarbonate co-transporter NBCe1-A (originally known as sodium bicarbonate cotransporter [NBC]), and the basolateral chloride bicarbonate exchanger AE2 [20–22]. While NHE3 is exclusively expressed by epididymal principal cells, NBCe1-A and AE2 are also present in narrow and clear cells. The apical sodium hydrogen exchanger, NHE2, has also been described in principal cells [23]. Interestingly, the level of expression of these transporters varies in different regions of the rat epididymis. For example, NHE3 is most abundant in the initial segments and proximal caput, and is not detected in the distal cauda [20], whereas NHE2 is absent from the initial segments [23]. Our laboratory has previously shown that apical acid extrusion following an acid load is reduced by approximately 50% in the presence of the Na/H exchanger inhibitors, EIPA and HOE694, in initial segment tubules isolated and perfused *in vitro* [20]. These results indicated that a pancreatic-like HOE694-sensitive NHE3 was expressed in these segments of the epididymis. Epididymal principal cells also have a high cytosolic carbonic anhydrase activity, and they express the membrane-associated carbonic anhydrases CAIV [19, 24, 25] and CAXIV [26] in their apical and basolateral membrane. Therefore, principal cells of the initial segments of the epididymis are fully equipped to achieve net bicarbonate reabsorption. Throughout the epididymis, clear cells participate in net proton secretion. In the distal regions, these cells become more numerous and their contribution to luminal acidification increases. The following sections describe selected aspects of vacuolar proton pumping ATPase (V-ATPase)-dependent proton secretion by clear cells in the distal portion of the epididymis and proximal vas deferens.

2 Expression of V-ATPase in clear cells

In the distal regions of the epididymis, luminal fluid is maintained at the acidic pH of 6.8 [8, 9]. Interestingly, the level of expression of the transporters that are involved in bicarbonate reabsorption in the proximal regions progressively diminishes towards the distal portions of the epididymis and the participation of clear cells in proton secretion appears to increase. As mentioned above, NHE3 is not expressed in the rat distal cauda epididymidis. In this epididymal region, the number of clear cells increases significantly (Figure 1) compared with the caput epididymidis. These cells are part of the "mitochondria-rich" cell family and are among a few specialized cell types that express the vacuolar proton pumping H^+-ATPase (V-ATPase) in their plasma membrane [27]. Whereas the V-ATPase is ubiquitously expressed and is responsible for the acidification of intracellular organelles in all cell types (reviewed in [28]), it is also expressed in the apical plasma membrane of acidifying cells, including epididymal clear cells [29–32] and renal intercalated cells [28], where it plays a key role in luminal acidification. Bafilomycin, a specific inhibitor of the V-ATPase, markedly reduces the rate of net proton secretion measured with an extracellular proton-selective electrode in cut-open vas deferens, a segment that also contains clear cells [29, 33, 34]. In addition, clear cells express the cytosolic carbonic anhydrase CAII (Figure 2) and the basolateral transporters NBCe1-A and AE2 [20–22, 29, 30, 33]. However, functional analysis shows that proton secretion by clear cells of the vas deferens is independent of chloride, while it is inhibited by disulphonic stilbenes (SITS), therefore identifying NBCe1-A as a potential player in this process [33]. In addition, the carbonic anhydrase inhibitor acetazolamide markedly reduces luminal acidification in the rat cauda epididymidis perfused *in vivo* [18], abolishes bafilomycin-sensitive net proton secretion in the cut-open vas deferens [33], and induces the internalization of V-ATPase in clear cells of the cauda epididymidis [11]. Therefore, the V-ATPase and CAII are key players

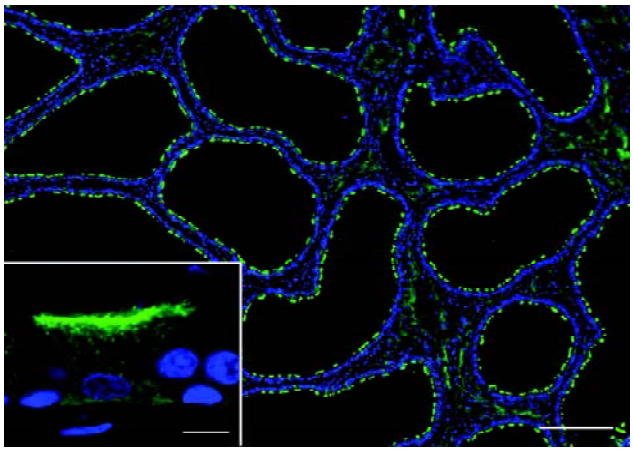

Figure 1. Cauda epididymidis perfused *in vivo* and labeled with anti-vacuolar proton pumping ATPase (V-ATPase) antibodies. Numerous clear cells, stained for the B1 subunit of the V-ATPase (green), are detected in the cauda epididymidis. Luminal spermatozoa are absent from these perfused tubules. Inset: high magnification of a clear cell showing V-ATPase staining in apical microvilli and sub-apical vesicles. Nuclei are stained in blue with DAPI. Bar = 150 μm. Inset: bar = 5 μm.

in luminal acidification by clear cells of the distal region of the epididymis and proximal vas deferens.

3 Plasticity of clear cells: cell shape changes and membrane insertion of the V-ATPase

Immunofluorescence and electron microscopy studies revealed that the V-ATPase is abundantly expressed in the apical pole of clear cells, where it is distributed between sub-apical vesicles and the apical plasma membrane (Figure 1 and Figure 2A) [11, 34, 35]. Functional data showed that proton secretion by these cells is regulated through dynamic V-ATPase recycling, and that an increase in V-ATPase plasma membrane expression is correlated with an increase in proton secretion [11, 34, 35]. Studies performed on the cauda epididymidis perfused *in vivo* showed that the sub-cellular localization of the V-ATPase is markedly modulated by the luminal environment [11, 35]. At the physiological pH of 6.8, the V-ATPase is distributed between apical microvilli and sub-apical endosomes. In contrast, when perfused with an alkaline solution (pH 7.8), the V-ATPase is mainly located in apical microvilli and very few sub-apical V-ATPase-labeled vesicles are detected. This accumulation of the V-ATPase in the plasma membrane, which occurs within 10–15 min, is accompanied by a significant elongation of apical microvilli that contain a high density of V-ATPase molecules [11]. Therefore, clear cells show a remarkable plasticity in response to physiological stimuli to increase their rate of V-ATPase-dependent proton secretion.

4 Soluble adenylyl cyclase (sAC) is a bicarbonate sensor that regulates the apical insertion of the V-ATPase

The mechanisms by which epithelial cells can respond to variations in the pH of their extracellular environment are still poorly understood. Our recent studies have identified the bicarbonate-activated adenylyl cyclase, sAC, as a key player in the response of clear cells to variations in pH and bicarbonate concentration in the rat epididymal lumen [11]. This enzyme is directly activated by bicarbonate ions but is not modulated by pH [12]. We have shown that sAC is enriched in clear cells from rat epididymides, compared with principal cells (Figure 3). Interestingly, acetazolamide completely inhibited the apical insertion of V-ATPase in response to luminal alkalinization, indicating that the production of intracellular bicarbonate by CAII is a key step in the targeting of the V-ATPase to the apical membrane [11]. In the presence of acetazolamide, clear cells show a complete internalization of the V-ATPase and no apical microvilli [11], and net bafilomycin-dependent proton secretion is completely abolished [33]. Furthermore, addition of luminal bicarbonate (12 mmol/L) at constant pH (7.1) induces a significant re-localization of the V-ATPase into well-developed microvilli, a response that is abolished by the sAC inhibitor, 2-hydroxyestradiol [11]. Finally, the cAMP permeant analogue, cpt-cAMP, mimics the bicarbonate and alkaline pH-induced V-ATPase apical accumulation. These results suggest that intracellular bicarbonate elevation following either an increase in luminal pH or direct addition of luminal bicarbonate activates sAC to produce cAMP, leading to the accumula-

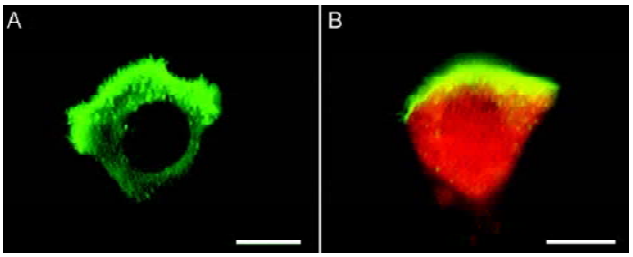

Figure 2. Human epididymis stained for the vacuolar proton pumping ATPase (V-ATPase) and carbonic anhydrase II (CAII). (A): Clear cell showing the presence of V-ATPase (subunit E) in apical microvilli and sub-apical vesicles (green). (B): Double-labeling for the V-ATPase (subunit E; green) and CAII (red). Abundant CAII is detected in the cytosol of a clear cell, which also expresses the V-ATPase in its apical pole. Bars = 5 μm.

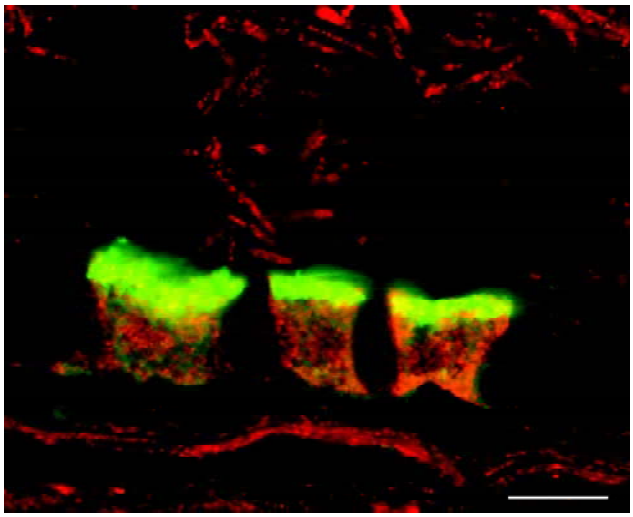

Figure 3. High expression of soluble adenylyl cyclase (sAC) in clear cells. Double labeling of rat cauda epididymidis for sAC (red) and vacuolar proton pumping ATPase (V-ATPase) (subunit E; green) reveals that clear cells (labeled in their apical pole for V-ATPase) contain abundant sAC throughout their cytoplasm. Spermatozoa and surrounding muscle cells are also stained for sAC. Bar = 10 μm. Reproduced with permission from J Biol Chem, Pastor-Soler *et al*. [11].

tion of the V-ATPase in the apical membrane of clear cells from the rat epididymis.

It is interesting to note that principal cells of the epididymis have the ability to secrete bicarbonate when activated by basolateral stimuli [36, 37]. This process depends on the presence of cystic fibrosis transmembrane conductance regulator (CFTR), located in the apical membrane of principal cells, which works in conjunction with a basolateral Na/H exchanger NHE1 [23, 38]. An acute increase in luminal bicarbonate concentration upon activation of the epithelium is proposed to "prime" spermatozoa prior to ejaculation [39]. However, this process would lead to an increase in luminal pH, which might be detrimental if maintained for a sustained period. We propose that clear cells respond to this rise in luminal bicarbonate by increasing their rate of proton secretion, after activation of sAC [11, 19]. A potential mode of entry for bicarbonate through the apical membrane might be the electro-neutral sodium bicarbonate co-transporter, NBC3 (also known as NBCn1), which has been described in the apical membrane of clear cells [40]. In this way, activation of clear cells by luminal bicarbonate would return the luminal pH to its resting acidic value.

5 Gelsolin participates in the regulation of V-ATPase recycling via modulation of the actin cytoskeleton

We have shown that modulation of the actin cytoskeleton is a key process in the regulation of V-ATPase trafficking and recycling in clear cells [35]. Some subunits of the V-ATPase, including subunits B1, B2 and C directly interact with actin [41–43]. In addition, subunit B1 also contains a C-terminal PDZ binding domain allowing it to associate with Na^+/H^+ exchanger regulatory factor 1 (NHERF1), a PDZ (PSD-95, *Drosophila* discs large protein, ZO-1) protein that directly interacts with actin-binding merlin-ezrin-radixin-moesin (MERM) proteins [44]. The actin cytoskeleton is under very dynamic remodeling in all cell types (reviewed in [45, 46]), including clear cells, and we have shown that the actin-capping and -severing protein, gelsolin, is highly expressed in these cells [35]. Modulation of the activity of gelsolin, using a permeant peptide that prevents its uncapping from the actin filament and promotes actin depolymerization, strongly increases the accumulation of V-ATPase in the plasma membrane of clear cells even at the acidic luminal pH of 6.5. Therefore, gelsolin-dependent actin depolymerization induces either inhibition of V-ATPase endocytosis or activation of exocytosis [35]. The severing property of gelsolin is dependent on calcium [47, 48]. Chelation of intracellular calcium by 1,2-bis (2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid tetrakis (acetoxymethyl ester, BAPTA-AM) inhibits the pH-induced apical V-ATPase accumulation [35]. In addition, the phospholipase C (PLC) inhibitor, U-73122 also abolishes this response [35]. We propose that maintenance of the actin cytoskeleton in a depolymerized state by gelsolin promotes calcium-dependent apical membrane accumulation of the V-ATPase in response to luminal alkaline pH conditions.

6 Male fertility and V-ATPase

The functional importance of the acidification capacity of the epididymis in male fertility was recently clearly demonstrated by the finding that male mice lacking the transcription factor Foxi1 are infertile [49]. Foxi1 is a major transcriptional regulator of several genes, including the B1 subunit of the V-ATPase and CAII. A more alkaline environment in the epididymal lumen, apparently due to the absence of both of these proteins, results in impaired sperm maturation, leading to the inability of spermatozoa to move up the female reproductive tract [49]. However, male mice that are deficient in a functional B1 subunit alone ($B1^{-/-}$), but continue to express CAII, are fertile, indicating that a compensatory mechanism is in place in these mice [50]. We have recently shown that the B2 subunit of the V-ATPase, which is normally expressed in intracellular structures, moves to the apical membrane of clear cells in $B1^{-/-}$ mice (Figure 4) [31]. The fact that the lumen of the epididymis of these mice is within the normal pH range confirms that the B2 subunit can compensate for the absence of B1. Interestingly, these mice do not develop metabolic acidosis when given a normal diet [50, 51], a phenotype that was expected owing to the high expression of B1-containing V-ATPase complexes in the apical membrane of proton-secreting renal intercalated cells of wild type mice (reviewed in [28]). Therefore, it appears that the B2 subunit also compensates for the absence of B1 in intercalated cells of $B1^{-/-}$ mice [52]. However, humans harboring single point mutations of the B1 subunit develop severe distal renal tubular acidosis (dRTA), indicating significant impairment of V-ATPase-dependent proton secretion in intercalated cells (reviewed in [28]). One possible explanation for this major difference in the phenotypes of $B1^{-/-}$ mice and humans with mutated B1 is that the impaired B1 subunit protein with single point mutations might assemble normally within the V-ATPase holoenzyme, therefore preventing a compensatory association of the B2 subunit. The mechanisms responsible for the insertion of the B2 subunit into the V-ATPase holoenzymes that are targeted to the plasma membrane in the absence of B1, compared with intrace-

llular targeting of B2-containing V-ATPases in the presence of B1, are under current investigation in our laboratory. It will be very interesting to determine whether or not humans harboring mutations of the B1 subunit are infertile. At the moment, most of these patients are juveniles and their fertility has not yet been assessed.

7 Conclusion

In summary, while significant bicarbonate reabsorption via principal cells occurs in the proximal regions of the epididymis, net proton secretion by clear cells is an important step in the establishment of a luminal acidic environment in the epididymis. The concerted action of various acid/base transporters localized in principal cells of the initial segments and caput (NHE2, NHE3, CAIV, CAXIV, NBCe1-A, and AE2) and in clear cells throughout the epididymis (V-ATPase, CAII, NBCe1-A, and AE2) is crucial to the establishment and maintenance of a low bicarbonate and low pH environment for the maturation of spermatozoa. Impairment of luminal acidification in the epididymis has important consequences for sperm maturation, which become unable to move up the female reproductive tract. Net proton secretion by clear cells is regulated through recycling of V-ATPase-containing vesicles to and from the apical membrane. The bicarbonate sensor, sAC, which is highly expressed in clear cells, is a crucial mediator of the response of these cells to variations in the bicarbonate concentration and pH of the luminal environment, at least in the rat epididymis. Dynamic modulation of the actin cytoskeleton by gelsolin, and an increase in intracellular calcium via the PLC-signaling pathway are also significant contributors to the regulation of V-ATPase-mediated net proton secretion in clear cells.

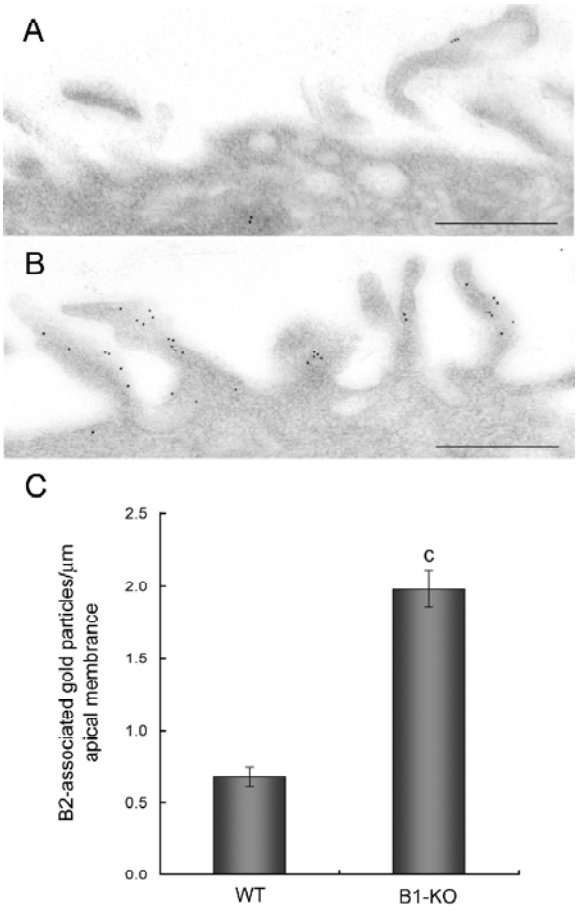

Figure 4. Electron microscopic immunogold labeling of the vacuolar proton pumping ATPase (V-ATPase) B2 subunit in the apical pole of a clear cell from the cauda epididymidis of a wild type (WT) mouse (A) and a $B1^{-/-}$ mouse (BI-KO) (B). The B2 subunit is mainly located in intracellular structures in wild type mouse clear cells (A) and predominant apical microvilli labeling is detected in clear cell from the $B1^{-/-}$ mouse (B). (C): Quantification analysis showed a significant increase in the number of B2-associated gold particles in the apical microvilli of clear cells from $B1^{-/-}$ mice compared to wild type mice ($^cP < 0.005$). Bars = 500 nm. Reproduced with permission from Am J Physiol Cell Physiol, Da Silva *et al*. [31].

Acknowledgment

This study was supported by National Institutes of Health (NIH) grant numbers HD40793 and DK38452 (to Dr Sylvie Breton). The Microscopy Core of the Massachusetts General Hospital Program in Membrane Biology received additional support from grant DK38452, from an NIH Center for the Study of Inflammatory Bowel Disease (DK43351) and an NIH Boston Area Diabetes and Endocrinology Research Center Grant (DK57521). Our special thanks go to Dr Dennis Brown for his critical reading of the manuscript and constructive comments.

References

1 Acott TS, Carr DW. Inhibition of bovine spermatozoa by caudal epididymal fluid: II. Interaction of pH and a quiescence factor. Biol Reprod 1984; 30: 926–35.
2 Jones RC, Murdoch RN. Regulation of the motility and metabolism of spermatozoa for storage in the epididymis of eutheran and marsupial mammals. Reprod Fertil Dev 1996; 8: 553–68.
3 Orgebin-Crist MC. The Epididymis in the Post-Genome Era. In: Hinton BT, Turner TT, editors. The Third International Conference on the Epididymis, vol. III. Charlottesville: The Van Doren Company; 2003: 2–22.
4 Yeung CH, Cooper TG, Oberpenning F, Schulze H, Nieschlag E. Changes in movement characteristics of human spermatozoa along the length of the epididymis. Biol Reprod 1993; 49: 274-80.
5 Hinton BT, Palladino MA. Epididymal epithelium: its contribution to the formation of a luminal fluid microenvironment.

Microsc Res Tech 1995; 30: 67–81.
6 Robaire B, Viger RS. Regulation of epididymal epithelial cell functions. Biol Reprod 1995; 52: 226–36.
7 Robaire B, Hermo L. Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. In: Knobil E. Neil J, editors. The Physiology of Reproduction. New York: Raven Press; 1988: 999–1080.
8 Levine N, Kelly H. Measurement of pH in the rat epididymis *in vivo*. J Reprod Fertil 1978; 52: 333–5.
9 Levine N, Marsh DJ. Micropuncture studies of the electrochemical aspects of fluid and electrolytes transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. J Physiol 1971; 213: 557–70.
10 Carr DW, Usselman MC, Acott TS. Effects of pH, lactate, and viscoelastic drag on sperm motility: a species comparison. Biol Reprod 1985; 33: 588–95.
11 Pastor-Soler N, Beaulieu V, Litvin TN, Da Silva N, Chen Y, Brown D, *et al*. Bicarbonate regulated adenylyl cyclase (sAC) is a sensor that regulates pH-dependent V-ATPase recycling. J Biol Chem 2003; 278: 49523–9.
12 Chen Y, Cann MJ, Litvin TN, Iourgenko V, Sinclair ML, Levin LR, *et al*. Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. Science 2000; 289: 625–8.
13 Sinclair ML, Wang XY, Mattia M, Conti M, Buck J, Wolgemuth DJ, *et al*. Specific expression of soluble adenylyl cyclase in male germ cells. Mol Reprod Dev 2000; 56: 6–11.
14 Visconti PE, Stewart-Savage J, Blasco A, Battaglia L, Miranda P, Kopf GS, *et al*. Roles of bicarbonate, cAMP, and protein tyrosine phosphorylation on capacitation and the spontaneous acrosome reaction of hamster sperm. Biol Reprod 1999; 61: 76–84.
15 Demarco IA, Espinosa F, Edwards J, Sosnik J, De La Vega-Beltran JL, Hockensmith JW, *et al*. Involvement of a Na^+/HCO-3 cotransporter in mouse sperm capacitation. J Biol Chem 2003; 278: 7001–9.
16 Hernandez-Gonzalez EO, Sosnik J, Edwards J, Acevedo JJ, Mendoza-Lujambio I, Lopez-Gonzalez I, *et al*. Sodium and epithelial sodium channels participate in the regulation of the capacitation-associated hyperpolarization in mouse sperm. J Biol Chem 2006; 281: 5623–33.
17 Kirichok Y, Navarro B, Clapham DE. Whole-cell patch-clamp measurements of spermatozoa reveal an alkaline-activated Ca^{2+} channel. Nature 2006; 439: 737–40.
18 Au CL, Wong PY. Luminal acidification by the perfused rat cauda epididymidis. J Physiol 1980; 309: 419–27.
19 Pastor-Soler N, Pietrement C, Breton S. Role of acid/base transporters in the male reproductive tract and potential consequences of their malfunction. Physiology (Bethesda) 2005; 20: 417–28.
20 Bagnis C, Marsolais M, Biemesderfer D, Laprade R, Breton S. Na^+/H^+-exchange activity and immunolocalization of NHE3 in rat epididymis. Am J Physiol Renal Physiol 2001; 280: F426–36.
21 Jensen LJ, Schmitt BM, Berger UV, Nsumu NN, Boron WF, Hediger MA, *et al*. Localization of sodium bicarbonate cotransporter (NBC) protein and messenger ribonucleic acid in rat epididymis. Biol Reprod 1999; 60: 573–9.
22 Jensen LJ, Stuart-Tilley AK, Peters LL, Lux SE, Alper SL, Breton S. Immunolocalization of AE2 anion exchanger in rat and mouse epididymis. Biol Reprod 1999; 61: 973–80.
23 Chew SB, Leung GP, Leung PY, Tse CM, Wong PY. Polarized distribution of NHE1 and NHE2 in the rat epididymis. Biol Reprod 2000; 62: 755–8.
24 Parkkila S, Parkkila AK, Kaunisto K, Waheed A, Sly WS, Rajaniemi H. Location of a membrane-bound carbonic anhydrase isoenzyme (CA IV) in the human male reproductive tract. J Histochem Cytochem 1993; 41: 751–7.
25 Kaunisto K, Parkkila S, Parkkila AK, Waheed A, Sly WS, Rajaniemi H. Expression of carbonic anhydrase isoenzymes IV and II in rat epididymal duct. Biol Reprod 1995; 52: 1350–7.
26 Hermo L, Chong DL, Moffatt P, Sly WS, Waheed A, Smith CE. Region- and cell-specific differences in the distribution of carbonic anhydrases II, III, XII, and XIV in the adult rat epididymis. J Histochem Cytochem 2005; 53: 699–713.
27 Brown D, Breton S. Mitochondria-rich, proton-secreting epithelial cells. J Exp Biol 1996; 199: 2345–58.
28 Wagner CA, Finberg KE, Breton S, Marshansky V, Brown D, Geibel JP. Renal vacuolar H^+-ATPase. Physiol Rev 2004; 84: 1263–314.
29 Breton S, Smith PJ, Lui B, Brown D. Acidification of the male reproductive tract by a proton pumping (H^+)-ATPase. Nat Med 1996; 2: 470–2.
30 Breton S, Tyszkowski R, Sabolic I, Brown D. Postnatal development of H^+ ATPase (proton-pump)-rich cells in rat epididymis. Histochem Cell Biol 1999; 111: 97–105.
31 Da Silva N, Shum WW, El-Annan J, Paunescu TG, McKee M, Smith PJ, *et al*. Relocalization of the V-ATPase B2 subunit to the apical membrane of epididymal clear cells of mice deficient in the B1 subunit. Am J Physiol Cell Physiol 2007; Epub ahead of print.
32 Hermo L, Adamali HI, Andonian S. Immunolocalization of CA II and H^+ V-ATPase in epithelial cells of the mouse and rat epididymis. J Androl 2000; 21: 376–91.
33 Breton S, Hammer K, Smith PJS, Brown D. Proton secretion in the male reproductive tract: involvement of chloride-independent bicarbonate transport. Am J Physiol Cell Physiol 1998; 275: C1134–42.
34 Breton S, Nsumu NN, Galli T, Sabolic I, Smith PJ, Brown D. Tetanus toxin-mediated cleavage of cellubrevin inhibits proton secretion in the male reproductive tract. Am J Physiol Renal Physiol 2000; 278: F717–25.
35 Beaulieu V, Da Silva N, Pastor-Soler N, Brown CR, Smith PJ, Brown D, *et al*. Modulation of the actin cytoskeleton via gelsolin regulates vacuolar H^+ ATPase (V-ATPase) recycling. J Biol Chem 2005; 280: 8452–63.
36 Wong PY. Mechanism of adrenergic stimulation of anion secretion in cultured rat epididymal epithelium. Am J Physiol 1988; 254: F121–33.
37 Sedlacek RL, Carlin RW, Singh AK, Schultz BD. Neurotransmitter-stimulated ion transport by cultured porcine vas deferens epithelium. Am J Physiol Renal Physiol 2001; 281: F557–70.
38 Wong PY. *CFTR* gene and male fertility. Mol Hum Reprod 1998; 4: 107–10.
39 Carlin RW, Lee JH, Marcus DC, Schultz BD. Adenosine stimulates anion secretion across cultured and native adult human vas deferens epithelia. Biol Reprod 2003; 68: 1027–34.
40 Pushkin A, Clark I, Kwon TH, Nielsen S, Kurtz I. Immunolocalization of NBC3 and NHE3 in the rat epididymis: colocalization of NBC3 and the vacuolar H^+-ATPase. J Androl 2000; 21: 708–20.
41 Vitavska O, Wieczorek H, Merzendorfer H. A novel role for subunit C in mediating binding of the H^+-V-ATPase to the actin cytoskeleton. J Biol Chem 2003; 278: 18499–505.
42 Holliday LS, Lu M, Lee BS, Nelson RD, Solivan S, Zhang L, *et al*. The amino-terminal domain of the B subunit of vacuolar H^+-ATPase contains a filamentous actin binding site. J Biol Chem 2000; 275: 32331–7.
43 Chen SH, Bubb MR, Yarmola EG, Zuo J, Jiang J, Lee BS, *et al*. Vacuolar H^+-ATPase binding to microfilaments: regulation in response to phosphatidylinositol 3-kinase activity and detailed characterization of the actin-binding site in subunit B. J Biol Chem 2004; 279: 7988–98.
44 Breton S, Wiederhold T, Marshansky V, Nsumu NN, Ramesh V, Brown D. The B1 subunit of the H^+ ATPase is a PDZ domain-

binding protein. Colocalization with NHE-RF in renal B-intercalated cells. J Biol Chem 2000; 275: 18219–24.
45 Apodaca G. Endocytic traffic in polarized epithelial cells: role of the actin and microtubule cytoskeleton. Traffic 2001; 2: 149–59.
46 Hoekstra D, Tyteca D, van IJzendoorn SC. The subapical compartment: a traffic center in membrane polarity development. J Cell Sci 2004; 117: 2183–92.
47 Janmey PA, Stossel TP. Modulation of gelsolin function by phosphatidylinositol 4,5-bisphosphate. Nature 1987; 325: 362–4.
48 Yin HL, Stossel TP. Control of cytoplasmic actin gel-sol transformation by gelsolin, a calcium-dependent regulatory protein. Nature 1979; 281: 583–6.
49 Blomqvist SR, Vidarsson H, Soder O, Enerback S. Epididymal expression of the forkhead transcription factor Foxi1 is required for male fertility. Embo J 2006; 25: 4131–41.
50 Finberg KE, Wagner CA, Bailey MA, Paunescu TG, Breton S, Brown D, *et al*. The B1-subunit of the H^+ ATPase is required for maximal urinary acidification. Proc Natl Acad Sci U S A 2005; 102: 13616–21.
51 Finberg K, Wang T, Wagner CA, Geibel JP, Dou H, Lifton RP. Generation and characterization of H^+-ATPase B1 subunit deficient mice. JASN 2001; 12: 3A (Abstract).
52 Paunescu T, Da Silva N, Marshansky V, McKee M, Breton S, Brown D. Expression of the 56kDa B2 subunit isoform of the vacuolar H^+-ATPase in proton secreting cells of the kidney and epididymis. Am J Physiol Cell Physiol 2004; 287: C149–62.