Title: Aquaporins and male (in)fertility

Summary sentence: Aquaporins, membrane channels widely expressed throughout the male reproductive tract, have roles in fluid balance and production of healthy and competent spermatozoa.

Keywords: Aquaporin; Male Reproduction; Spermatozoa; Testis; Water Transport.

Authors and affiliations: David F. Carrageta[1], Raquel L. Bernardino[1], Graça Soveral[2,3], Giuseppe Calamita[4], Marco G. Alves[1], Pedro F. Oliveira[1,5,6]✉[1]

[1] Department of Microscopy, Laboratory of Cell Biology, Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal

[2] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

[3] Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

This work was supported by "Fundação para a Ciência e a Tecnologia" - FCT to David F. Carrageta (SFRH/BD/136779/2018). The work was co-funded by FEDER through the COMPETE/QREN, FSE/POPH to Marco G. Alves (IFCT2015 and PTDC/MEC-AND/28691/2017); Pedro F. Oliveira (IFCT2015 and PTDC/BBB-BQB/1368/2014); UMIB (PEst-OE/SAU/UI0215/2019); co-funded by the EU Framework Programme for Research and Innovation H2020 (POCI/COMPETE2020).

✉**Corresponding author:** Pedro F. Oliveira, PhD

Department of Microscopy, Institute for Biomedical Sciences Abel Salazar, University of Porto.

Rua de Jorge Viterbo Ferreira n.228, 4050-313 Porto, Portugal.

Phone: +351 220428000 E-mail:pfobox@gmail.com

[4] Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari "Aldo Moro", Bari, Italy

[5] i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

[6] Department of Genetics, Faculty of Medicine, University of Porto, Porto, Portugal

Abstract

Aquaporins (AQPs) are a family of transmembrane channel proteins responsible not only for the transport of water but also small uncharged molecules. The discovery of AQPs revolutionized the study of physiological water transport and, currently, AQPs are regarded as pivotal for both, tissue and cellular fluid homeostasis. Thirteen distinct isoforms have been identified in mammals (AQP0-12), being roughly classified into three main groups based on their homology for substrates and biophysical properties of molecular transport. Throughout the male reproductive tract, AQPs greatly enhance water transport across all biological barriers, providing a constant and expeditious movement of water and playing an active role in the regulation of water and ion homeostasis. This regulation of fluids is particularly important in the male reproductive tract, where proper fluid composition is directly linked with a healthy and competent spermatozoa production. For instance, in the testis, fluid regulation is essential for spermatogenesis and posterior spermatozoa transport into the epididymal ducts, while maintaining proper ionic conditions for their maturation and storage. On the other hand, alterations in the expression pattern of AQPs or their dysfunction is linked with male subfertility and infertility. Thus, AQPs are of considerable importance for male reproductive health. In this review, we will discuss the most recent data on the expression and function of the different AQPs isoforms in the human, mouse and rat male reproductive tract. In addition, the regulation of AQPs expression and dysfunction linked with male infertility will be discussed along with their potential pharmacological value.

1. Introduction

Aquaporins (AQPs) are a family of transmembrane channel proteins responsible for the transport of water and a series of small uncharged molecules such as glycerol [1], urea [2], ammonia [3], hydrogen peroxide [4], some metalloids [5], small metabolites, like lactate and certain gases across biological membranes [6]. The discovery of AQPs in 1992, by Peter Agre and collaborators, revolutionized the study of cellular water transport. For the first time, a protein acting as a selective water pore was identified and characterized, not only in mammals but also in plants (Maurel et al, EMBO J 1993_PMID 8508761) and bacteria (Calamita et al, JBC 1995_PMID 7493926). Meritoriously, Agre was laureated in 2003 with the Nobel Prize in Chemistry for his detailed study of the structure and function of AQPs [7]. Since then, thirteen distinct isoforms have been identified (AQP0-12) in mammals and grossly (Pedro this classification is by now outdated as it does not suit the real transport functions of the AQPs) classified into three main groups based on their homology and biophysical properties (Figure 1). Interestingly, all AQPs are tetramers of four pores, where each monomer establishes an independent pore. However, this tetrameric structure also assembles a fifth central pore, which is described with hydrophobic nature and whose size and biophysical properties vary among groups and even among isoforms of the same group [8]. The first group is constituted by the orthodox AQPs. Seven AQPs belong in the orthodox group: AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8. Orthodox AQPs are described as selective to water molecules, although there are few exceptions. For instance, AQP6 was reported to be involved in the transport of Cl^- under acidic conditions [9] and AQP8 has a role in the transport of ammonia and hydrogen peroxide, which

justify the designation of ammoniaporin or peroxiporin [10, 11]. The second group concerns to aquaglyceroporins and includes four homologues: AQP3, AQP7, AQP9, and AQP10. Aquaglyceroporins have a bigger pore size [12] and are permeable not only to water but also to small uncharged solutes, such as glycerol, urea, or hydrogen peroxide [13, 14]. The last group is constituted by the so-called unorthodox AQPs, AQP11 and AQP12, also known as superaquaporins. Superaquaporins present low homology in comparison to orthodox AQPs and aquaglyceroporins [15]. Moreover, superaquaporins are often localized in the membrane of intracellular organelles. Thus, it has been suggested that the role of AQP11 and AQP12 is mainly linked with intracellular water and glycerol transport, regulating organelles volume and homeostasis [16-18]. Further work is needed to fully understand the properties and biological relevance of AQP11 and AQP12.

The transport of water through biological membranes is a vital process in cellular physiology, not only for tissue fluid homeostasis but also for intracellular processes. For instance, the regulation of water homeostasis is particularly important in the male reproductive tract for a healthy and competent spermatozoa production [19, 20]. In the testis, fluid regulation is essential for spermatogenesis and posterior transport of spermatozoa into the epididymal ducts, while maintaining proper conditions for their maturation. In the seminiferous tubules, fluid homeostasis is mainly regulated by Sertoli cells and partly by differentiating germ cells [21]. Indeed, it is estimated that 70% of cell volume is osmotically eliminated from the cytoplasm of spermatids during their differentiation into spermatozoa [22]. Subsequently, the maturation, concentration and storage of spermatozoa are associated with the secretion and absorption of fluid [23-25]. In this sense, AQPs greatly enhance water transport across all biological barriers, including in the male reproductive tract, while providing a constant and expeditious movement of water across tight junction barriers and playing an active role in the epithelial regulation of water homeostasis [26]. In this review, we will discuss the most recent data concerning the expression and function of the different AQPs isoforms in the human and rodent (mouse and rat) male reproductive tract.

In addition, the regulation of their expression and the association with male (in)fertility will be discussed.

2. Aquaporins expression and functions throughout the male reproductive tract

AQPs are widely expressed in the human male reproductive tract (Table 1). However, the data available concerning some isoforms are not consistent or even scarce. The presence of AQPs greatly differs among tissue regions and cell types, evidencing a complex regulatory and functional network. Besides, AQPs expression is generally disparate between mammals, with a few exceptions. Curiously, rodents (such as mouse and rat) share some similarities with humans, which has proven to be helpful as the majority of functional and knockout studies have been conducted in these species. Yet, only the first steps have been taken in order to elucidate the full extent of AQPs expression and function in human reproductive system. On the following topics, the most recent data concerning AQPs expression in the human reproductive system will be discussed, highlighting the putative function obtained from studies in humans and also rodent animal models.

2.1. Aquaporin 0 (AQP0)

Although not yet identified in the testicular environment of humans, the major intrinsic protein (MIP) of lens fiber, also known as AQP0, is predicted to be expressed in the human testis [27]. As an orthodox isoform, AQP0 is reported to mediate mostly water transport, although at a lower extent, when compared with other orthodox AQPs [28]. Besides, AQP0 is also reported to act as a cell-to-cell adhesion protein in the human eye, more properly in the lens fiber cells. In this tissue, AQP0 dysfunction is associated with cataractogenesis, both in humans and in knockout mice models [29, 30]. However, the consequences for the male reproductive system are unknown as it was not studied in these knockout mice models [30]. Nonetheless, Hermo *et al.* [31] identified

AQP0 in rat testis, more specifically in Leydig and Sertoli cells. In this study, the authors reported that the expression in Sertoli cells seems to be region-specific and stage-dependent, more specifically in a semicircular pattern at stages VI-VIII of the spermatogenic cycle. These results suggest a significant role in the transport of water into the lumen of the seminiferous tubules during specific stages of spermatogenesis, which are related to the release of elongating spermatids. Hence, AQP0 may improve the movement of spermatozoa into the epididymis by transporting water into seminiferous tubules. Interestingly, AQP0 is reported to be modulated by pH and Ca^{2+} [32, 33], which are tightly regulated in the testis and suggests that AQP0 might be important for proper fluid homeostasis in the seminiferous tubules.

2.2. Aquaporin-1 (AQP1)

AQP1 was in fact the first water channel discovered, while also the first described to act as a gas channel [34, 35]. AQP1 is widely expressed in humans, where it functions as a major water transporter. Besides, AQP1 is reported as crucial for angiogenesis, cell migration and cell growth [36]. AQP1 is expressed in the human testis, although restricted to the endothelial cells of blood vessels [37]. On the other hand, AQP1 is absent in germ cells and ejaculated spermatozoa [38]. Outside the testis, AQP1 was found in noncilitated epithelial cells of human efferent ducts and epididymis [39]. Based on its expression, it has been suggested that AQP1 plays a major role in the re-absorption of water to increase sperm concentration. Similarly to what was seen in humans, AQP1 is mainly expressed in rat efferent ducts and epididymis [40, 41], but not on murine spermatozoa [42]. In addition, AQP1 was also identified in the *rete testis*, *vas deferens*, prostate, and seminal vesicles from mouse [42].

2.3. Aquaporin-2 (AQP2)

AQP2 is an arginine vasopressin-regulated AQP that is exclusively permeable to water. AQP2 is widely expressed in the kidney and it is well known that mutations on the gene encoding AQP2 lead to severe forms of nephrogenic diabetes insipidus [43]. Thus, it is suggested that AQP2 main function specifies in water reabsorption in the kidney. However, AQP2 might not be specific for the kidney as it has been also found to be expressed in the human epididymis and seminal vesicles [44].

In rodents, the expression of AQP2 is rather distinct. In mouse, AQP2 was found in the epithelial cells of the *vas deferens* and seminiferous tubules [45]. In rats, Stevens *et al.* [46] found that AQP2 is expressed in the ampulla of the *vas deferens* [46]. This pattern of expression led the authors to suggest that AQP2, similarly to AQP1, may have a role in water absorption in order to increase sperm concentration [46]. Besides, Arrighi *et al.* [41] identified AQP2 in the *cauda* of the epididymis of young rats and its expression appears to decrease with the transition into adulthood, which suggests a role in the post-natal development.

2.4. Aquaporin-3 (AQP3)

AQP3 is an aquaglyceroporin that is widely expressed in humans. AQP3 is reported to be permeable to several molecules besides water, such as glycerol, urea [6], and hydrogen peroxide [47, 48]. In the human reproductive system, AQP3 was identified at the basolateral membranes of the prostatic epithelium and in the seminiferous tubules [49]. In addition, AQP3 was predicted to be expressed in human seminal vesicles and epididymis [44]. In human spermatozoa, AQP3 is not only present but is also considered essential for its physiology and function. AQP3 is found in spermatozoa's tail membrane and in its absence the male gamete exhibits alterations in volume regulation and excessive cell swelling when in the female reproductive tract [50, 51]. Therefore, as upon ejaculation spermatozoa experiences an osmotic decrease within the transition to the female reproductive tract, AQP3 was found to be crucial for spermatozoa osmoregulation.

Besides, AQP3 is reported to be responsive to the pH [52], further highlighting its function in osmoregulation and fluid dynamics.

In mouse, AQP3 was also identified in the midpiece of spermatozoa [51]. Moreover, AQP3 was identified in mouse testis [53, 54]. More recently, we also identified AQP3 expression in the TM4 cells, a cell line derived from a primary culture of mouse Sertoli cells [54]. In that study, the authors reported that not only AQP3 is expressed in mouse Sertoli cells, but also that it is important for the transport of glycerol in these cells. In rats, AQP3 was found to be expressed in the prostate [55] and in the epididymis, more specifically in the basal cells, suggesting a role in the transport of water and glycerol to the epididymal lumen during sperm maturation [31].

2.5. Aquaporin-4 (AQP4)

Although present, limited data are available concerning the expression of AQP4 in the human male reproductive tract. Data from microarrays identified the expression of AQP4 in human seminiferous tubules, seminal vesicles, prostate and epididymis [56]. In rats, AQP4 was identified in the prostate [55] and Sertoli cells [57]. Interestingly, our group was able to report that AQP4 physically interacts with CFTR, which suggests that AQP4 may play a role in water and ion homeostasis in seminiferous tubule and consequently in the constitution of seminiferous tubular fluid ionic content [57].

2.6. Aquaporin-5 (AQP5)

In a similar mode to what happens with AQP4, the data available on the presence and function of AQP5 in the human male reproductive system is quite scarce. AQP5 is predicted to be present in the human testis, more specifically in germ cells and Leydig cells [44]. In rats, AQP5 is only present in the *corpus* and *cauda* of epididymis, but the relevance for male reproductive potential is not known yet [58].

196

197 **2.7. Aquaporin-6 (AQP6)**

198 Contrariwise to other orthodox AQPs, the function of AQP6 is rather distinct. AQP6 is reported
199 to be colocalized with the H^+-ATPase in intracellular vesicles in the kidney, but not in the plasma
200 membrane, suggesting that AQP6 may act as an intra-vesicle pH regulator [59]. Interestingly, in
201 this same study Yasui and collaborators reported that AQP6 seems to be permeable to water only
202 at acidic conditions or in the presence of $HgCl_2$. Additionally, AQP6 is reported to transport urea,
203 glycerol [60], and nitrate [61], which led some authors to classify this isoform as an unorthodox
204 AQP. Nonetheless, there is no evidence that AQP6 is expressed in the human male reproductive
205 tract. The sole evidence was obtained in rats, with *AQP6* mRNA transcripts being identified in
206 whole epididymis lysates, while its protein was not detected in the epididymal epithelial cells
207 [58].

208

209 **2.8. Aquaporin-7 (AQP7)**

210 AQP7 is an aquaglyceroporin that is able to mediate the transport of water, glycerol, urea [62],
211 ammonia, and arsenite [5]. AQP7 is mostly known for its expression in the adipose tissue, where
212 it is reported to mediate the efflux of lipolytic glycerol **[63, 64]**. Herein, abnormal regulation of
213 glycerol transport is associated with the development of metabolic disease, highlighting that an
214 AQP7 dysfunction may be associated with obesity [65, 66] **(here I would also cite the review**
215 **Rodriguez et al 2015_PMID 26594198)**.

216 In the human reproductive system, Saito *et al.* [67] identified AQP7 in the testis, more specifically
217 in round and elongated spermatids, and in the tail of spermatozoa. Conversely, different studies
218 reported different patterns of expression in human spermatozoa. While Moretti *et al.* [68] reported
219 that AQP7 was indeed present in the tail and midpiece, Laforenza *et al.* [69] reported presence of
220 AQP7 only in the head of human spermatozoa. In fact, there is evidence that different patterns of

expression or even the absence of AQP7 in human spermatozoa are linked with abnormal morphology and decreased motility, and thus infertility, which will be further discussed.

In rats, AQP7 was identified in the seminiferous epithelium [62], round and elongated spermatids, residual bodies [70], and in the epididymis [58, 71]. Due to its pattern of expression, it was suggested that AQP7 may play a role in testis development **(to insert ref 70, here)** and in the reduction of cytoplasm during spermiogenesis [62]. Besides, AQP7 expression in the male reproductive tract was also reported to vary with age. Unlike AQP2, AQP7 expression seems to increase with the transition to adulthood [70]. In mouse, our group identified the expression of *Aqp7* mRNA in the testis, although no expression was detected in Sertoli cells [54].

2.9. Aquaporin-8 (AQP8)

AQP8, a homologue with permeability to ammonia and hydrogen peroxide besides that of water, was firstly identified in the intracellular domains of the proximal tubule and the collecting ducts cells [72]. Although it is expressed in the plasma membrane, AQP8 is also found in the mitochondrial membrane **(here, to insert Ferri et al Hepatology_PMID 14512882)**[73], where, at least in hepatocytes, it is suggested facilitates the transport of hydrogen peroxide [4, 48] and ammonia **(Soria et al Hepatology_PMID 23299935**) rather than water **(Calamita et al J Endocrinol_PMID 17210748**).

In the human male reproductive system, AQP8 appears to be expressed in the cytoplasmic compartment (in intracellular organelles or vesicles) of Sertoli cells and of spermatogonia and spermatids [38, 74]. The presence of this aquaporin in the plasma membrane was observed all germ cells and ejaculated spermatozoa, more specifically in the tail and midpiece [38]. Interestingly, in this same study the authors reported that the expression of AQP8 was transversal to all donors and it was not correlated with motility. As mature spermatozoa exhibits an increased mitochondrial activity, and thus high reactive oxygen species and hydrogen peroxide production [75], it was postulated that AQP8 facilitates their efflux minimizing the oxidative stress damage.

Similarly to the human, in rat testes, AQP8 is expressed in primary spermatocytes, elongated spermatids, Sertoli cells, and residual bodies [70, 76]. In fact, the expression of AQP8 in the plasma membrane of Sertoli cells was found at all stages of the seminiferous epithelium cycle suggesting relevance for AQP8 in fluid homeostasis in this epithelium [76, 77]. However, its expression in the epididymis is still inconclusive [72, 76]. Like AQP7, AQP8 has been suggested to exert a role in the reduction of cytoplasm during spermiogenesis. Additionally, its expression in the testes also seems to vary with age, increasing from 15 to 20 post-natal days [70, 78]. In mouse, few data are available on AQP8 expression and relevance in the male reproductive tract. This AQP was described in the mouse testis, more specifically in the residual cytoplasm of elongated spermatids [23]. No functional data are available.

2.10. Aquaporin-9 (AQP9)

AQP9 has been the focus of intensive research over the last years. AQP9 is thought to have a main role in transporting water, glycerol, and other solutes essential for sperm production and maturation [79]. AQP9 has been also reported to permeable to monocarboxylic acids, such as lactic acid and acetic acid, probably due to its larger pore size [80]. In the human reproductive system, AQP9 was found with low expression in the germinal epithelium, spermatocytes and Sertoli cells [38]. As lactate is a vital energetic substrate for germ cells and spermatogenesis [81], the presence of AQP9 in Sertoli cells indicates that this channel may help in the transport of lactate to the tubular fluid, sharing this role with the high expression of monocarboxylate transporters (MCTs) [82]. In addition, AQP9 was identified in the *vas deferens*, efferent ducts, more specifically on the apical membrane of nonciliated cells, and principal cells of the epididymis [83]. Based on its pattern of expression in this tissue, AQP9 is tought to play a role in fluid reabsorption and homeostasis. On the other hand, AQP9 is reported to be absent in human spermatozoa [38].

With some similarities with humans, AQP9 is reported to be expressed in Sertoli cells [84], Leydig cells, efferent ducts, all regions of epididymis, *vas deferens*, prostate, and coagulating gland of rats [76, 83]. In fact, there is evidence that AQP9 expression is cell-specific in the testis and region-specific in the efferent ducts and epididymis [76]. In mouse, our group recently identified the expression of AQP9 in both testis and Sertoli cells [54]. Additionally, we showed that AQP9 is the major aquaglyceroporin expressed in mouse Sertoli cells, where this isoform is essential to regulate the transport of water and glycerol. While widely distributed in the male reproductive tract of rodents, its expression in spermatozoa is still a matter of debate. Although AQP9 transcripts were found in mouse spermatozoa, immunoblotting did not confirm the result [23].

2.11. Aquaporin-10 (AQP10)

AQP10 is an aquaglyceroporin which seems to be specifically expressed in the human gastrointestinal tract and adipocytes [85, 86]. AQP10 is mainly responsible for water and glycerol transport, being highlighted in pair with AQP7 as a major glycerol transporter of adipocytes [87]. However, data concerning AQP10 in male reproductive tissues are still scarce. There is no evidence that AQP10 is expressed in the human male reproductive tract. In rats, AQP10 is expressed in the efferent ducts and epididymis [31]. Conversely, AQP10 is a pseudogene in mice and may lead to proteins without functional activity [88].

2.12. Aquaporin-11 (AQP11) and Aquaporin-12 (AQP12)

AQP11 is a relatively recent discovered isoform that has been classified as a superaquaporin. AQP11 is described as a water and glycerol channel that is mainly expressed in the vicinity of lipid droplets and in the endoplasmic reticulum membrane [18]. Few studies also addressed AQP11 expression and function in the human male reproductive system. *AQP11* mRNA was identified in human testicular tissue [89]. Using immunocytochemistry techniques Laforenza *et*

298 *al.* [69] identified this channel in granules and vesicles of soma and in the plasma membrane of
299 the tail of ejaculated human spermatozoa. Hence, the subcellular localization of AQP11 remains
300 debatable.

301 In rats, AQP11 was identified in the epididymis and testes [71]. Interestingly, Hermo *et al.* [90]
302 reported that AQP11 expression varies with age, as this transmembrane protein expression was
303 observed to decrease from young to adult rats. Additionally, AQP11 was identified in rat
304 elongated spermatids and mouse testes [89]. However, as knockout *Aqp11* animal models are not
305 viable due to the development of polycystic kidney and subsequent premature death due kidney
306 failure [91], few studies addressed AQP11 in the male reproductive tract and new methodologies
307 are needed to further investigate the role of this superaquaporin in the reproductive health.
308 Concerning AQP12, this isoform is reported to be exclusively expressed in acinar cells of the
309 pancreas and, thus, absent from the male reproductive tract [92].

310

311 **3. Alteration of aquaporins expression is linked with male infertility**

312 As aforementioned, AQPs are widely distributed throughout the male reproductive tract and in
313 male gametes. Their main functions comprise absorption/secretion dynamics in order to maintain
314 the homeostasis of the reproductive system to produce healthy and functional spermatozoa.
315 Specific expression patterns in cells, regions, variation with age, and evidence of compensatory
316 mechanisms are indicators of a precise and complex mechanism of regulation and function.
317 Besides, AQPs also play a role in accessory glands, contributing to the composition of seminal
318 fluid [42, 83], and in spermatozoa adaptation phenomena after ejaculation, due to the variation of
319 osmotic conditions [50]. Thus, AQPs are of extreme importance for the reproductive health and
320 their dysfunction is related to reproductive disorders.

321 Although data are scarce, there is evidence suggesting that alterations in the expression and
322 function of these transport proteins are associated with subfertility or infertility [93]. Association
323 between a deficit in AQP7 expression in spermatozoa and diminished quality and fertilizing

ability has been reported. In humans, it was found that spermatozoa from infertile individuals' presents lower amounts or were devoid of AQP7. In addition, spermatozoa lacking AQP7 presented significantly lower motility in comparison with spermatozoa exhibiting positive AQP7 staining [38, 67]. In another study, Moretti *et al.* [68] also reported that AQP7 expression was diminished in morphologically abnormal spermatozoa. On the other hand, there are studies with a knockout *Aqp7* animal model that may jeopardize those findings. Knockout mice for *Aqp7* were described as fertile, without differences in testis morphology and with healthy functional spermatozoa [94]. Moreover, other study with knockout mice for *Aqp7* reported that an upregulation of *Aqp8* expression might have a compensatory effect [23]. Hence, further research is necessary to elucidate the role of AQP7 in the male reproductive health.

Like AQP7, alterations in AQP3 and AQP8 expression were associated with reduced reproductive capability. It was found that a deficit of AQP3 led to impaired sperm motility and tail deformations [51]. Concerning AQP8, although few studies addressed its expression and function in spermatozoa, it has been reported that its expression is inversely correlated with the coiling degree of the tail, suggesting that this AQP may play a role in the adaptation to osmolality variations [38]. Additionally, Laforenza *et al.* [69] proposed that AQP8 malfunction could lead to hydrogen peroxide accumulation in the mitochondria, hence affecting sperm function. Still, no differences in AQP8 expression were found between fertile and infertile human individuals [38] and it was reported that knockout *Aqp8* animal models remain fertile, which suggests a functional compensation [95]. Thus, the importance of AQP8 for the reproductive health requires further investigation.

3.1. Hormonal regulation of aquaporins

Hormonal alterations are a known factor linked with reduced male reproductive health and those alterations might be associated with altered AQPs expression and function. For instance, there is evidence that AQP1 and AQP9 are regulated by estrogens. In studies with knockout mice for

estrogen receptor α (*Erα)* it was found a reduced expression of AQP1 and AQP9 in the epididymis and efferent ducts, suggesting that estrogens may regulate the fluid absorption in these ducts. These animals also presented a fluid accumulation in efferent ducts with consequently testicular atrophy and infertility [96, 97]. In addition, in a recent study by our group, we found that AQP9 is downregulated by increased estrogen levels in mouse Sertoli cells [54]. Besides, it was also reported that the administration of estrogens to rats reduces the expression of AQP9 in the epididymis, but these effects were reversed with the administration of testosterone [98]. Still in the epididymis, it has been reported that orchiectomized rats were devoid of AQP3 in basal epididymal cells. However, when testosterone was administrated a slightly restoration of AQP3 expression was noticed, suggesting that testosterone can modulate the expression of AQP3 in the epididymis [31]. Curiously, similar effects were also reported for AQP9 expression [99], suggesting that estrogens would decrease specific isoforms of AQPs expression while testosterone administration would attenuate those effects. Nevertheless, there are contradictory data. Studies showed that while androgens do modulate AQP9 in the initial segment of the epididymis, estrogens did not alter its expression [100], or that estrogens administration increased AQP9 expression in efferent ducts [101]. These inconsistencies in the literature illustrate the complexity surrounding the regulation of AQPs. In sum, while there is evidence to support that AQPs are under hormonal regulation, few data are available and further research on the topic is required.

3.2. Interactions between AQPs and CFTR

Cystic fibrosis transmembrane conductance regulator, or CFTR, is a transmembrane channel protein responsible for the transport of Cl^- and HCO_3^-, regulating the ionic balance and pH in the tubular fluid [102, 103]. Mutations in CFTR are correlated with cystic fibrosis, a pathology related with infertility scenarios. CFTR is widely expressed and performs an essential role in the male reproductive tract, which requires a tight regulation of water and electrolytes in order to produce

healthy spermatozoa. In fact, mutations in *CFTR* can lead to anatomical abnormalities of the reproductive tract, mainly a congenital bilateral absence of the vas deferens and, consequently, infertility [104]. Although CFTR is not permeable to water, this protein acts as a regulator of other protein channels, such as AQPs. In fact, CFTR acts as a molecular partner of AQPs in epithelial cells, regulating fluid homeodynamics. For instance, our group described the expression of CFTR, AQP4 and AQP9 in cultured rat primary Sertoli cells [57, 84]. In this study we also investigated the physical interaction between CFTR and AQP4 and AQP9 in Sertoli cells. Taking advantage of co-immunoprecipitation techniques, we observed a molecular interaction between CFTR and both AQP4 and AQP9. Since AQP4 and AQP9 are reported as crucial for water and ion homeostasis in several tissues [23, 38, 98], it is also expectable that both these AQPs regulate water homeostasis in Sertoli cells and consequently inside the seminiferous tubules. Taken together, these results support the hypothesis that CFTR is, at least in Sertoli cells, a regulator of AQPs activity in water homeodynamics. Additionally, there is further evidence that CFTR interacts with AQP9. Both CFTR and AQP9 are co-localized in the luminal membrane of the principal cells in the epididymis of rat and humans [105, 106]. In fact, *in vivo* and *in vitro* inhibition of both AQP9 and CFTR reduced water permeability [105]. Thus, it has been suggested that CFTR may potentiate water permeability of AQP9 in the epididymal epithelium, which is essential for proper sperm maturation and movement. On a side note, it was also described that AQP7 and AQP8 are expressed with in a highly similar pattern with CFTR in the testis, highlighting a possible interaction [93, 107]. In support of this hypothesis, CFTR was co-localized with several other AQPs in different tissues, such as AQP1 and AQP5 in the pancreas [108]. Notwithstanding, it is still unclear how CFTR physically regulates AQPs function.

3.3. Relevance of AQPs in obesity and metabolic diseases

Obesity and metabolic syndrome have also been associated with impaired male reproductive function [82, 109, 110]. During obesity scenarios, there is not only an increased aromatization of

testosterone to estradiol [111, 112], but also an increased plasmatic concentration of free fatty acids and glycerol (for review [113]). High glycerol concentrations are responsible for the disruption of the homeostasis of the tubular fluid due to the impaired function of the blood-testis barrier, leading to the apoptosis of germ cells. Thus, while acute exposition to high concentration of glycerol could lead to temporary arrest of spermatogenesis, chronic exposition may result in permanent oligospermia or even azoospermia [114, 115]. Since aquaglyceroporins are responsible for the transport of glycerol, their function or expression might be altered in cases of obesity or, more in general in the metabolic syndrome. In fact, high-fat diet was already associated with increased AQP9 and decreased AQP1 expression in the epididymis [116]. In another study, Pei *et al.* [55] reported a decreased expression of AQP1, AQP3 and AQP4 in the prostate of diabetic rats. Still, much of the role of AQPs in male reproductive tract in obesity and metabolic syndrome cases is unknown and an interesting matter of investigation.

3.4. Varicocele and possible role of AQPs

It is worth mentioning that AQPs differential expression is also associated with varicocele. When comparing healthy individuals with those who suffer from idiopathic varicocele, it was found that AQP9 expression was reduced or absent in the primary spermatocytes and germ cells of the later **(of the later?)** [117]. Moreover, AQP1 was identified in Sertoli and germ cells of individuals suffering from varicocele, in opposition to the absence of this AQP in healthy individuals [39].

4. Conclusion and future perspectives

Since the discover of AQPs, the perspective on water transport has been revolutionized. AQPs are now the matter of intense research worldwide, where the elucidation of patterns of expression and function may lead to new therapeutic approaches for several diseases. Herein, we highlight that AQPs are widely distributed through the male reproductive tract, where these transmembrane

proteins are vital for a healthy spermatogenesis and the maintenance of fluid homeostasis. Expression patterns were found to be specific to cell and tissue regions, or even age of the individual, indicating a precise and complex mechanism of regulation and function. Indeed, AQPs are of extreme importance for the reproductive health and their malfunction is related to reproductive disorders. Nevertheless, the available data are not always consistent and few are the studies directly addressing the pathogenetic relevance of these channels in the male reproductive tract. In this sense, knockout animal models provide valuable evidence, despite of some differences among species. In sum, AQPs pattern of expression may indeed constitute one important biomarker for male reproductive health. Further studies are essential to clarify the role of AQPs in male fertility and how these channel proteins may be targets for future therapeutic interventions.

Conflicts of interest

The authors declare no conflicts of interests.

References

1. Borgnia MJ, Agre P. Reconstitution and functional comparison of purified GlpF and AqpZ, the glycerol and water channels from Escherichia coli. Proceedings of the National Academy of Sciences of the United States of America 2001; 98:2888-2893.

2. Litman T, Sogaard R, Zeuthen T. Ammonia and urea permeability of mammalian aquaporins. Handbook of Experimental Pharmacology 2009:327-358.

3. Saparov SM, Liu K, Agre P, Pohl P. Fast and selective ammonia transport by aquaporin-8. Journal of Biological Chemistry 2007; 282:5296-5301.

4. Bienert GP, Moller AL, Kristiansen KA, Schulz A, Moller IM, Schjoerring JK, Jahn TP. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. Journal of Biological Chemistry 2007; 282:1183-1192.

5. Liu Z, Shen J, Carbrey JM, Mukhopadhyay R, Agre P, Rosen BP. Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9. Proceedings of the National Academy of Sciences of the United States of America 2002; 99:6053-6058.

6. Bernardino RL, Marinelli RA, Maggio A, Gena P, Cataldo I, Alves MG, Svelto M, Oliveira PF, Calamita G. Hepatocyte and Sertoli Cell Aquaporins, Recent Advances and Research Trends. International Journal of Molecular Sciences 2016; 17.

7. Agre P. Aquaporin water channels (Nobel Lecture). Angewandte Chemie International Edition in English 2004; 43:4278-4290.

8. Ozu M, Galizia L, Acuna C, Amodeo G. Aquaporins: More Than Functional Monomers in a Tetrameric Arrangement. Cells 2018; 7:209.

9. Yasui M. pH regulated anion permeability of aquaporin-6. Handbook of Experimental Pharmacology 2009:299-308.

10. Soria LR, Fanelli E, Altamura N, Svelto M, Marinelli RA, Calamita G. Aquaporin-8-facilitated mitochondrial ammonia transport. Biochemical and Biophysical Research Communications 2010; 393:217-221.

11. Chauvigne F, Boj M, Finn RN, Cerda J. Mitochondrial aquaporin-8-mediated hydrogen peroxide transport is essential for teleost spermatozoon motility. Scientific Reports 2015; 5:7789.

12. Sales AD, Lobo CH, Carvalho AA, Moura AA, Rodrigues AP. Structure, function, and localization of aquaporins: their possible implications on gamete cryopreservation. Genetics and Molecular Research 2013; 12:6718-6732.

13. Watanabe S, Moniaga CS, Nielsen S, Hara-Chikuma M. Aquaporin-9 facilitates membrane transport of hydrogen peroxide in mammalian cells. Biochemical and Biophysical Research Communications 2016; 471:191-197.

14. Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S. Aquaporin water channels--from atomic structure to clinical medicine. Journal of Physiology 2002; 542:3-16.

15. Gorelick DA, Praetorius J, Tsunenari T, Nielsen S, Agre P. Aquaporin-11: a channel protein lacking apparent transport function expressed in brain. BMC Biochemistry 2006; 7:14.

16. Nozaki K, Ishii D, Ishibashi K. Intracellular aquaporins: clues for intracellular water transport? Pflügers Archiv : European Journal of Physiology 2008; 456:701-707.

17. Badaut J, Fukuda AM, Jullienne A, Petry KG. Aquaporin and brain diseases. Biochimica et Biophysica Acta 2014; 1840:1554-1565.

18. Madeira A, Fernandez-Veledo S, Camps M, Zorzano A, Moura TF, Ceperuelo-Mallafre V, Vendrell J, Soveral G. Human aquaporin-11 is a water and glycerol channel and localizes in the vicinity of lipid droplets in human adipocytes. Obesity (Silver Spring) 2014; 22:2010-2017.

19. Levine N, Marsh DJ. Micropuncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. Journal of Physiology 1971; 213:557-570.

20. Russell LD, Bartke A, Goh JC. Postnatal-Development of the Sertoli-Cell Barrier, Tubular Lumen, and Cytoskeleton of Sertoli and Myoid Cells in the Rat, and Their Relationship to Tubular Fluid Secretion and Flow. American Journal of Anatomy 1989; 184:179-189.

21. Sprando RL, Russell LD. Comparative study of cytoplasmic elimination in spermatids of selected mammalian species. American Journal of Anatomy 1987; 178:72-80.

22. Cho YS, Svelto M, Calamita G. Possible functional implications of aquaporin water channels in reproductive physiology and medically assisted procreation. Cellular and Molecular Biology (Noisy-le-Grand, France) 2003; 49:515-519.

23. Yeung CH, Callies C, Rojek A, Nielsen S, Cooper TG. Aquaporin isoforms involved in physiological volume regulation of murine spermatozoa. Biology of Reproduction 2009; 80:350-357.

24. Yeung CH, Barfield JP, Cooper TG. Chloride channels in physiological volume regulation of human spermatozoa. Biology Reproduction 2005; 73:1057-1063.

25. Yeung CH, Barfield JP, Cooper TG. The role of anion channels and Ca2+ in addition to K+ channels in the physiological volume regulation of murine spermatozoa. Molecular Reproduction and Development 2005; 71:368-379.

26. Alves MG, Sa R, Jesus TT, Sousa M, Oliveira PF. CFTR Regulation of Aquaporin-Mediated Water Transport: A Target in Male Fertility. Current Drug Targets 2015; 16:993-1006.

27. Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K, Asplund A, Sjostedt E, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Molecular & Cellular Proteomics 2014; 13:397-406.

28. Chandy G, Zampighi GA, Kreman M, Hall JE. Comparison of the water transporting properties of MIP and AQP1. Journal of Membrane Biology 1997; 159:29-39.

29. Kumari SS, Gandhi J, Mustehsan MH, Eren S, Varadaraj K. Functional characterization of an AQP0 missense mutation, R33C, that causes dominant congenital lens cataract, reveals impaired cell-to-cell adhesion. Experimental Eye Research 2013; 116:371-385.

30. Shiels A, Bassnett S, Varadaraj K, Mathias R, Al-Ghoul K, Kuszak J, Donoviel D, Lilleberg S, Friedrich G, Zambrowicz B. Optical dysfunction of the crystalline lens in aquaporin-0-deficient mice. Physiological Genomics 2001; 7:179-186.

521 31. Hermo L, Krzeczunowicz D, Ruz R. Cell specificity of aquaporins 0, 3, and 10 expressed
522 in the testis, efferent ducts, and epididymis of adult rats. Journal of Andrology 2004; 25:494-505.

523 32. Kalman K, Nemeth-Cahalan KL, Froger A, Hall JE. AQP0-LTR of the Cat Fr mouse
524 alters water permeability and calcium regulation of wild type AQP0. Biochimica et Biophysica
525 Acta 2006; 1758:1094-1099.

526 33. Kalman K, Nemeth-Cahalan KL, Froger A, Hall JE. Phosphorylation determines the
527 calmodulin-mediated Ca2+ response and water permeability of AQP0. Journal of Biological
528 Chemistry 2008; 283:21278-21283.

529 34. Nakhoul NL, Davis BA, Romero MF, Boron WF. Effect of expressing the water channel
530 aquaporin-1 on the CO2 permeability of Xenopus oocytes. American Journal of Physiology-Cell
531 Physiology 1998; 274:C543-548.

532 35. Prasad GV, Coury LA, Finn F, Zeidel ML. Reconstituted aquaporin 1 water channels
533 transport CO2 across membranes. Journal of Biological Chemistry 1998; 273:33123-33126.

534 36. Nico B, Ribatti D. Aquaporins in tumor growth and angiogenesis. Cancer Letters 2010;
535 294:135-138.

536 37. Nicotina PA, Romeo C, Arena S, Impellizzeri P, Antonuccio P, Arena F, Zuccarello B,
537 Romeo G. Immunohistology of aquaporin-1 and stem cell factor-receptor in human undescended
538 testes. Pediatric Surgery International 2004; 20:271-275.

539 38. Yeung CH, Callies C, Tuttelmann F, Kliesch S, Cooper TG. Aquaporins in the human
540 testis and spermatozoa - identification, involvement in sperm volume regulation and clinical
541 relevance. International Journal of Andrology 2010; 33:629-641.

542 39. Nicotina PA, Romeo C, Arena S, Arena F, Maisano D, Zuccarello B. Immunoexpression
543 of aquaporin-1 in adolescent varicocele testes: possible significance for fluid reabsorption.
544 Urology 2005; 65:149-152.

545 40. Brown D, Verbavatz JM, Valenti G, Lui B, Sabolic I. Localization of the CHIP28 water
546 channel in reabsorptive segments of the rat male reproductive tract. European Journal of Cell
547 Biology 1993; 61:264-273.

548 41. Arrighi S, Aralla M, Genovese P, Picabea N, Bielli A. Undernutrition during foetal to
549 prepubertal life affects aquaporin 9 but not aquaporins 1 and 2 expression in the male genital tract
550 of adult rats. Theriogenology 2010; 74:1661-1669.

551 42. Lu DY, Li Y, Bi ZW, Yu HM, Li XJ. Expression and immunohistochemical localization
552 of aquaporin-1 in male reproductive organs of the mouse. Anatomia, Histologia, Embryologia
553 2008; 37:1-8.

554 43. Agre P, Kozono D. Aquaporin water channels: molecular mechanisms for human
555 diseases. FEBS Letters 2003; 555:72-78.

556 44. Lizio M, Harshbarger J, Shimoji H, Severin J, Kasukawa T, Sahin S, Abugessaisa I,
557 Fukuda S, Hori F, Ishikawa-Kato S, Mungall CJ, Arner E, et al. Gateways to the FANTOM5
558 promoter level mammalian expression atlas. Genome Biology 2015; 16:22.

559 45. Nelson RD, Stricklett P, Gustafson C, Stevens A, Ausiello D, Brown D, Kohan DE.
560 Expression of an AQP2 Cre recombinase transgene in kidney and male reproductive system of
561 transgenic mice. American Journal of Physiology-Cell Physiology 1998; 275:C216-226.

562 46. Stevens AL, Breton S, Gustafson CE, Bouley R, Nelson RD, Kohan DE, Brown D.
563 Aquaporin 2 is a vasopressin-independent, constitutive apical membrane protein in rat vas
564 deferens. American Journal of Physiology-Cell Physiology 2000; 278:C791-802.

565 47. Almasalmeh A, Krenc D, Wu B, Beitz E. Structural determinants of the hydrogen
566 peroxide permeability of aquaporins. FEBS Journal 2014; 281:647-656.

567 48. Bienert GP, Chaumont F. Aquaporin-facilitated transmembrane diffusion of hydrogen
568 peroxide. Biochimica et Biophysica Acta 2014; 1840:1596-1604.

569 49. Mobasheri A, Wray S, Marples D. Distribution of AQP2 and AQP3 water channels in
570 human tissue microarrays. Journal of Molecular Histology 2005; 36:1-14.

571 50. Chen Q, Duan EK. Aquaporins in sperm osmoadaptation: an emerging role for volume
572 regulation. Acta Pharmacologica Sinica 2011; 32:721-724.

573 51. Chen Q, Peng H, Lei L, Zhang Y, Kuang H, Cao Y, Shi QX, Ma T, Duan E. Aquaporin3
574 is a sperm water channel essential for postcopulatory sperm osmoadaptation and migration. Cell
575 Research 2011; 21:922-933.

576 52. de Almeida A, Martins AP, Mosca AF, Wijma HJ, Prista C, Soveral G, Casini A.
577 Exploring the gating mechanisms of aquaporin-3: new clues for the design of inhibitors?
578 Molecular BioSystems 2016; 12:1564-1573.

579 53. Ma T, Song Y, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Nephrogenic
580 diabetes insipidus in mice lacking aquaporin-3 water channels. Proceedings of the National
581 Academy of Sciences of the United States of America 2000; 97:4386-4391.

582 54. Bernardino RL, Carrageta DF, Silva AM, Calamita G, Alves MG, Soveral G, Oliveira
583 PF. Estrogen Modulates Glycerol Permeability in Sertoli Cells through Downregulation of
584 Aquaporin-9. Cells 2018; 7:153.

585 55. Pei L, Yang G, Jiang J, Jiang R, Deng Q, Chen B, Gan X. Expression of aquaporins in
586 prostate and seminal vesicles of diabetic rats. Journal of Sexual Medicine 2013; 10:2975-2985.

587 56. Mobasheri A, Marples D, Young IS, Floyd RV, Moskaluk CA, Frigeri A. Distribution of
588 the AQP4 water channel in normal human tissues - Protein and tissue microarrays reveal
589 expression in several new anatomical locations, including the prostate gland and seminal vesicles.
590 Channels 2007; 1:30-39.

591 57. Jesus TT, Bernardino RL, Martins AD, Sa R, Sousa M, Alves MG, Oliveira PF.
592 Aquaporin-4 as a molecular partner of cystic fibrosis transmembrane conductance regulator in rat
593 Sertoli cells. Biochemical and Biophysical Research Communications 2014; 446:1017-1021.

58. Da Silva N, Silberstein C, Beaulieu V, Pietrement C, Van Hoek AN, Brown D, Breton S. Postnatal expression of aquaporins in epithelial cells of the rat epididymis. Biology of Reproduction 2006; 74:427-438.

59. Yasui M, Hazama A, Kwon TH, Nielsen S, Guggino WB, Agre P. Rapid gating and anion permeability of an intracellular aquaporin. Nature 1999; 402:184-187.

60. Holm LM, Klaerke DA, Zeuthen T. Aquaporin 6 is permeable to glycerol and urea. Pflugers Archiv-European Journal of Physiology 2004; 448:181-186.

61. Ikeda M, Beitz E, Kozono D, Guggino WB, Agre P, Yasui M. Characterization of aquaporin-6 as a nitrate channel in mammalian cells - Requirement of pore-lining residue threonine 63. Journal of Biological Chemistry 2002; 277:39873-39879.

62. Ishibashi K, Kuwahara M, Gu Y, Kageyama Y, Tohsaka A, Suzuki F, Marumo F, Sasaki S. Cloning and functional expression of a new water channel abundantly expressed in the testis permeable to water, glycerol, and urea. Journal of Biological Chemistry 1997; 272:20782-20786.

63. Madeira A, Camps M, Zorzano A, Moura TF, Soveral G. Biophysical Assessment of Human Aquaporin-7 as a Water and Glycerol Channel in 3T3-L1 Adipocytes. Plos One 2013; 8:e83442.

64. Mosca AF, de Almeida A, Wragg D, Martins AP, Sabir F, Leoni S, Moura TF, Prista C, Casini A, Soveral G. Molecular Basis of Aquaporin-7 Permeability Regulation by pH. Cells 2018; 7:207.

65. Hibuse T, Maeda N, Funahashi T, Yamamoto K, Nagasawa A, Mizunoya W, Kishida K, Inoue K, Kuriyama H, Nakamura T, Fushiki T, Kihara S, et al. Aquaporin 7 deficiency is associated with development of obesity through activation of adipose glycerol kinase. Proceedings of the National Academy of Sciences of the United States of America 2005; 102:10993-10998.

618 66. Hara-Chikuma M, Sohara E, Rai T, Ikawa M, Okabe M, Sasaki S, Uchida S, Verkman
619 AS. Progressive adipocyte hypertrophy in aquaporin-7-deficient mice - Adipocyte glycerol
620 permeability as a novel regulator of fat accumulation. Journal of Biological Chemistry 2005;
621 280:15493-15496.

622 67. Saito K, Kageyama Y, Okada Y, Kawakami S, Kihara K, Ishibashi K, Sasaki S.
623 Localization of aquaporin-7 in human testis and ejaculated sperm: possible involvement in
624 maintenance of sperm quality. Journal of Urology 2004; 172:2073-2076.

625 68. Moretti E, Terzuoli G, Mazzi L, Iacoponi F, Collodel G. Immunolocalization of
626 aquaporin 7 in human sperm and its relationship with semen parameters. Systems Biology in
627 Reproductive Medicine 2012; 58:129-135.

628 69. Laforenza U, Pellavio G, Marchetti AL, Omes C, Todaro F, Gastaldi G. Aquaporin-
629 Mediated Water and Hydrogen Peroxide Transport Is Involved in Normal Human Spermatozoa
630 Functioning. International Journal of Molecular Sciences 2016; 18.

631 70. Calamita G, Mazzone A, Bizzoca A, Svelto M. Possible involvement of aquaporin-7 and
632 -8 in rat testis development and spermatogenesis. Biochemical and Biophysical Research
633 Communications 2001; 288:619-625.

634 71. Hermo L, Schellenberg M, Liu LY, Dayanandan B, Zhang T, Mandato CA, Smith CE.
635 Membrane domain specificity in the spatial distribution of aquaporins 5, 7, 9, and 11 in efferent
636 ducts and epididymis of rats. Journal of Histochemistry and Cytochemistry 2008; 56:1121-1135.

637 72. Elkjaer ML, Nejsum LN, Gresz V, Kwon TH, Jensen UB, Frokiaer J, Nielsen S.
638 Immunolocalization of aquaporin-8 in rat kidney, gastrointestinal tract, testis, and airways.
639 American Journal of Physiology-Renal Physiology 2001; 281:F1047-1057.

640 73. Calamita G, Ferri D, Gena P, Liquori GE, Cavalier A, Thomas D, Svelto M. The inner
641 mitochondrial membrane has aquaporin-8 water channels and is highly permeable to water.
642 Journal of Biological Chemistry 2005; 280:17149-17153.

643 74. Rato L, Socorro S, Cavaco JE, Oliveira PF. Tubular fluid secretion in the seminiferous
644 epithelium: ion transporters and aquaporins in Sertoli cells. Journal of Membrane Biology 2010;
645 236:215-224.

646 75. Dias TR, Alves MG, Silva BM, Oliveira PF. Sperm glucose transport and metabolism in
647 diabetic individuals. Molecular and Cellular Endocrinology 2014; 396:37-45.

648 76. Badran HH, Hermo LS. Expression and regulation of aquaporins 1, 8, and 9 in the testis,
649 efferent ducts, and epididymis of adult rats and during postnatal development. Journal of
650 Andrology 2002; 23:358-373.

651 77. Tani T, Koyama Y, Nihei K, Hatakeyama S, Ohshiro K, Yoshida Y, Yaoita E, Sakai Y,
652 Hatakeyama K, Yamamoto T. Immunolocalization of aquaporin-8 in rat digestive organs and
653 testis. Archives of Histology and Cytology 2001; 64:159-168.

654 78. Calamita G, Mazzone A, Cho YS, Valenti G, Svelto M. Expression and localization of
655 the aquaporin-8 water channel in rat testis. Biology of Reproduction 2001; 64:1660-1666.

656 79. Yeste M, Morato R, Rodriguez-Gil JE, Bonet S, Prieto-Martinez N. Aquaporins in the
657 male reproductive tract and sperm: Functional implications and cryobiology. Reproduction in
658 Domestic Animals 2017; 52 Suppl 4:12-27.

659 80. Badaut J, Regli L. Distribution and possible roles of aquaporin 9 in the brain.
660 Neuroscience 2004; 129:971-981.

661 81. Alves MG, Martins AD, Rato L, Moreira PI, Socorro S, Oliveira PF. Molecular
662 mechanisms beyond glucose transport in diabetes-related male infertility. Biochimica et
663 Biophysica Acta 2013; 1832:626-635.

664 82. Rato L, Alves MG, Socorro S, Duarte AI, Cavaco JE, Oliveira PF. Metabolic regulation
665 is important for spermatogenesis. Nature Reviews Urology 2012; 9:330-338.

666 83. Pastor-Soler N, Bagnis C, Sabolic I, Tyszkowski R, McKee M, Van Hoek A, Breton S,
667 Brown D. Aquaporin 9 expression along the male reproductive tract. Biology of Reproduction
668 2001; 65:384-393.

669 84. Jesus TT, Bernardino RL, Martins AD, Sa R, Sousa M, Alves MG, Oliveira PF.
670 Aquaporin-9 is expressed in rat Sertoli cells and interacts with the cystic fibrosis transmembrane
671 conductance regulator. IUBMB Life 2014; 66:639-644.

672 85. Ishibashi K, Morinaga T, Kuwahara M, Sasaki S, Imai M. Cloning and identification of
673 a new member of water channel (AQP10) as an aquaglyceroporin. Biochimica Et Biophysica
674 Acta-Gene Structure and Expression 2002; 1576:335-340.

675 86. Laforenza U, Scaffino MF, Gastaldi G. Aquaporin-10 represents an alternative pathway
676 for glycerol efflux from human adipocytes. PLoS One 2013; 8:e54474.

677 87. Gotfryd K, Mosca AF, Missel JW, Truelsen SF, Wang KT, Spulber M, Krabbe S, Helix-
678 Nielsen C, Laforenza U, Soveral G, Pedersen PA, Gourdon P. Human adipose glycerol flux is
679 regulated by a pH gate in AQP10. Nature Communications 2018; 9:4749.

680 88. Morinaga T, Nakakoshi M, Hirao A, Imai M, Ishibashi K. Mouse aquaporin 10 gene
681 (AQP10) is a pseudogene. Biochemical and Biophysical Research Communications 2002;
682 294:630-634.

683 89. Yeung CH, Cooper TG. Aquaporin AQP11 in the testis: molecular identity and
684 association with the processing of residual cytoplasm of elongated spermatids. Reproduction
685 2010; 139:209-216.

686 90. Hermo L, Smith CE. Thirsty business: cell, region, and membrane specificity of
687 aquaporins in the testis, efferent ducts, and epididymis and factors regulating their expression.
688 Journal of Andrology 2011; 32:565-575.

689 91. Matsuzaki T, Yaguchi T, Shimizu K, Kita A, Ishibashi K, Takata K. The distribution and
690 function of aquaporins in the kidney: resolved and unresolved questions. Anatomical Science
691 International 2017; 92:187-199.

692 92. Itoh T, Rai T, Kuwahara M, Ko SB, Uchida S, Sasaki S, Ishibashi K. Identification of a
693 novel aquaporin, AQP12, expressed in pancreatic acinar cells. Biochemical and Biophysical
694 Research Communications 2005; 330:832-838.

695 93. Huang HF, He RH, Sun CC, Zhang Y, Meng QX, Ma YY. Function of aquaporins in
696 female and male reproductive systems. Human Reproduction Update 2006; 12:785-795.

697 94. Sohara E, Ueda O, Tachibe T, Hani T, Jishage K, Rai T, Sasaki S, Uchida S. Morphologic
698 and functional analysis of sperm and testes in Aquaporin 7 knockout mice. Fertility and Sterility
699 2007; 87:671-676.

700 95. Yang B, Song Y, Zhao D, Verkman AS. Phenotype analysis of aquaporin-8 null mice.
701 American Journal of Physiology-Cell Physiology 2005; 288:C1161-1170.

702 96. Zhou Q, Clarke L, Nie R, Carnes K, Lai LW, Lien YH, Verkman A, Lubahn D, Fisher
703 JS, Katzenellenbogen BS, Hess RA. Estrogen action and male fertility: roles of the
704 sodium/hydrogen exchanger-3 and fluid reabsorption in reproductive tract function. Proceedings
705 of the National Academy of Sciences of the United States of America 2001; 98:14132-14137.

706 97. Weiss J, Bernhardt ML, Laronda MM, Hurley LA, Glidewell-Kenney C, Pillai S, Tong
707 M, Korach KS, Jameson JL. Estrogen actions in the male reproductive system involve estrogen
708 response element-independent pathways. Endocrinology 2008; 149:6198-6206.

709 98. Pastor-Soler NM, Fisher JS, Sharpe R, Hill E, Van Hoek A, Brown D, Breton S.
710 Aquaporin 9 expression in the developing rat epididymis is modulated by steroid hormones.
711 Reproduction 2010; 139:613-621.

712 99. Pastor-Soler N, Isnard-Bagnis C, Herak-Kramberger C, Sabolic I, Van Hoek A, Brown
713 D, Breton S. Expression of aquaporin 9 in the adult rat epididymal epithelium is modulated by
714 androgens. Biology of Reproduction 2002; 66:1716-1722.

715 100. Oliveira CA, Carnes K, Franca LR, Hermo L, Hess RA. Aquaporin-1 and -9 are
716 differentially regulated by oestrogen in the efferent ductule epithelium and initial segment of the
717 epididymis. Biology of the Cell 2005; 97:385-395.

718 101. Wellejus A, Jensen HE, Loft S, Jonassen TE. Expression of aquaporin 9 in rat liver and
719 efferent ducts of the male reproductive system after neonatal diethylstilbestrol exposure. Journal
720 of Histochemistry & Cytochemistry 2008; 56:425-432.

721 102. Bernardino RL, Jesus TT, Martins AD, Sousa M, Barros A, Cavaco JE, Socorro S, Alves
722 MG, Oliveira PF. Molecular basis of bicarbonate membrane transport in the male reproductive
723 tract. Current Medicinal Chemistry 2013; 20:4037-4049.

724 103. Xu WM, Chen J, Chen H, Diao RY, Fok KL, Dong JD, Sun TT, Chen WY, Yu MK,
725 Zhang XH, Tsang LL, Lau A, et al. Defective CFTR-dependent CREB activation results in
726 impaired spermatogenesis and azoospermia. PLoS One 2011; 6:e19120.

727 104. de Souza DAS, Faucz FR, Pereira-Ferrari L, Sotomaior VS, Raskin S. Congenital
728 bilateral absence of the vas deferens as an atypical form of cystic fibrosis: reproductive
729 implications and genetic counseling. Andrology 2018; 6:127-135.

730 105. Cheung KH, Leung CT, Leung GP, Wong PY. Synergistic effects of cystic fibrosis
731 transmembrane conductance regulator and aquaporin-9 in the rat epididymis. Biology of
732 Reproduction 2003; 68:1505-1510.

733 106. Ruz R, Andonian S, Hermo L. Immunolocalization and regulation of cystic fibrosis
734 transmembrane conductance regulator in the adult rat epididymis. Journal of Andrology 2004;
735 25:265-273.

736 107. Suzuki-Toyota F, Ishibashi K, Yuasa S. Immunohistochemical localization of a water
737 channel, aquaporin 7 (AQP7), in the rat testis. Cell and Tissue Research 1999; 295:279-285.

738 108. Burghardt B, Elkaer ML, Kwon TH, Racz GZ, Varga G, Steward MC, Nielsen S.
739 Distribution of aquaporin water channels AQP1 and AQP5 in the ductal system of the human
740 pancreas. Gut 2003; 52:1008-1016.

741 109. Oliveira PF, Sousa M, Silva BM, Monteiro MP, Alves MG. Obesity, energy balance and
742 spermatogenesis. Reproduction 2017; 153:R173-R185.

743 110. Alves MG, Jesus TT, Sousa M, Goldberg E, Silva BM, Oliveira PF. Male fertility and
744 obesity: are ghrelin, leptin and glucagon-like peptide-1 pharmacologically relevant? Current
745 Pharmaceutical Design 2016; 22:783-791.

746 111. Schneider G, Kirschner MA, Berkowitz R, Ertel NH. Increased estrogen production in
747 obese men. Journal of Clinical Endocrinology and Metabolism 1979; 48:633-638.

748 112. Cohen P. The hypogonadal–obesity cycle: role of aromatase in modulating the
749 testosterone–estradiol shunt–a major factor in the genesis of morbid obesity. Medical Hypotheses
750 1999; 52:49-51.

751 113. Crisostomo L, Alves MG, Calamita G, Sousa M, Oliveira PF. Glycerol and testicular
752 activity: the good, the bad and the ugly. Molecular Human Reproduction 2017; 23:725-737.

753 114. Wiebe JP, Kowalik A, Gallardi RL, Egeler O, Clubb BH. Glycerol disrupts tight junction-
754 associated actin microfilaments, occludin, and microtubules in Sertoli cells. Journal of Andrology
755 2000; 21:625-635.

756 115. Wiebe JP, Barr KJ, Buckingham KD. Sustained azoospermia in squirrel monkey, Saimiri
757 sciureus, resulting from a single intratesticular glycerol injection. Contraception 1989; 39:447-
758 457.

116. Marchiani S, Vignozzi L, Filippi S, Gurrieri B, Comeglio P, Morelli A, Danza G, Bartolucci G, Maggi M, Baldi E. Metabolic syndrome-associated sperm alterations in an experimental rabbit model: relation with metabolic profile, testis and epididymis gene expression and effect of tamoxifen treatment. Molecular and Cellular Endocrinology 2015; 401:12-24.

117. Arena S, Arena F, Maisano D, Di Benedetto V, Romeo C, Nicotina PA. Aquaporin-9 immunohistochemistry in varicocele testes as a consequence of hypoxia in the sperm production site. Andrologia 2011; 43:34-37.

Figure Legend

Figure 1 – Aquaporins classification based on their homology and biophysical properties. Currently, thirteen distinct isoforms have been identified in mammals (AQP0-12) and grossly classified into three main groups: orthodox, aquaglyceroporins and superaquaporins. Although high homology exists among homologues of the same group, each homologue may exhibit a different specificity in its permeability to solutes.

778 **Table 1 –** Male reproductive tract distribution and suggested functions of all aquaporin isoforms.

Isoform	Localization	Putative function in male reproduction	References
AQP0	**Human** testis* **Rat** Leydig and Sertoli cells	Homeostasis of the tubular fluid; transport of water into the lumen of seminiferous tubules	[27], [31]
AQP1	**Human** testis, efferent ducts and epididymis **Rat** efferent ducts and epididymis **Mouse** rete testis, vas deferens, prostate, and seminal vesicles	Absorption of water and regulation of sperm concentration	[37], [39], [40], [41], [42]
AQP2	**Human** epididymis* and seminal vesicles* **Rat** vas deferens and cauda of epididymis **Mouse** vas deferens and seminiferous tubules	Absorption of water and regulation of sperm concentration; possible role in post-natal development	[41], [44], [45], [46]
AQP3	**Human** prostate, seminiferous tubules, seminal vesicles*, epididymis*, and spermatozoa **Rat** epididymis and prostate **Mouse** Sertoli cells and spermatozoa	Transport of water and glycerol to the epididymal lumen; regulation of spermatozoa volume and osmoadaptation in the uterine cavity	[44], [49], [50], [51], [53], [54], [55]
AQP4	**Human** seminiferous tubules, seminal vesicles, prostate, and epididymis **Rat** prostate and Sertoli cells	Water and ionic homeostasis	[55], [56], [57]
AQP5	**Human** testis*, germ cells*, and Leydig cells* **Rat** corpus and cauda of epididymis	Transport of water and small molecules	[44], [58]
AQP6	No evidence of expression	-	[58]

AQP7	**Human** testis, round and elongated spermatids, and spermatozoa **Rat** seminiferous epithelium, round and elongated spermatids, residual bodies, and epididymis	Transport of water, glycerol and small molecules; reduction of the cytoplasm during spermatogenesis; sperm motility	[58], [62], [67], [68], [69], [70], [71]
AQP8	**Human** germ cells, Sertoli cells, spermatids, and spermatozoa; **Rat** testis, primary spermatocytes, elongated spermatids, Sertoli cells, and residual bodies	Tubular fluid homeostasis; reduction of cytoplasm during spermiogenesis; release of hydrogen peroxide accumulated in spermatozoa mitochondria	[38], [70], [74], [76], [77], [78]
AQP9	**Human** germ cells, spermatocytes, Sertoli cells, vas deferens, efferent ducts, and epididymis **Rat** Sertoli cells, Leydig cells, efferent ducts, epididymis, vas deferens, and prostate **Mouse** testis and Sertoli cells	Water and glycerol transport; fluid reabsorption; possible role in the transport of lactate to the tubular fluid	[38], [54], [77], [81], [82], [83], [84]
AQP10	**Rat** efferent ducts and epididymis	Water and glycerol transport	[31]
AQP11	**Human** testis and spermatozoa **Rat** epididymis, testis, and elongated spermatids **Mouse** testis	Water and glycerol transport	[69], [71], [89], [90]
AQP12	No evidence of expression	-	[92]

779 *Predicted based on mRNA microarrays