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# Aquaporin in mammalian species: A review

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**Abstract**
The discovery of water specific channels answered the longstanding biophysical and biochemical question relating water transport across different biological membranes and at the molecular level provided a full insight into the fundamental physiology of water balance and the pathophysiology associated with its dysfunction. Data revealing the presence of Aquaporin (AQP) in sperm is scanty, but most of its isoforms except AQP6 and AQP12 are found in different organs including testes, efferent ducts and epididymis. Presence of mainly AQP1 and AQP9 in the efferent duct plays a crucial role in the reabsorption/secretion dynamics of the luminal fluid during maturation process and transport of the sperm. Localization of other isoforms namely AQP3, AQP7, AQP8 and AQP11 has been confirmed in the sperm where it is found to be involved in volume regulation, which is required for the differentiation of spermatids into spermatozoa during spermiogenesis process as well as facilitates sperm transit along environments of different osmolarity (male and female reproductive tract). In the present review we cover the mechanisms underlying regulation of this protein and the associated pathophysiology which will throw a light for new research ventures regarding the manipulation of this protein for sequential management of disorders associated with water balance.

**Keywords:** Aquaporin (AQP), mammals, localization, regulation, dysfunction

## Introduction
Suitable concentrations of water and solutes are prerequisites for proper cell functioning and survivability. So, it is essential for the right substances to enter cells and waste substances to be eliminated. Attempts to identify the water channel proteins were essentially frozen in time for many years. It ensured limited success when researchers tried to isolate the protein from purified membranes of erythrocytes in toad bladder and kidney, although some publications came close (Benga *et al*., 1986) [6] and others provided important clues, such as information about the molecular size of the protein about 30 kD (Van Hoek *et al*., 1991) [48].

Biological membranes have an intrinsic, but limited, water permeability which can be explained by their lipid composition. Indeed, water penetrates slowly through this lipid bilayer by simple diffusion, due to the hydrophobic nature of the lipid bilayer (Matsuzaki *et al*., 2002) [29]. However, it was hard to explain water permeability in cells, such as erythrocytes, renal tubular epithelial cells or gametes (cells with higher metabolic activity) if water only passed through the plasma membrane via simple diffusion. Considering these facts, assumption was made that water should mainly flow through cell membranes by a faster mechanism which involves passive transport rather than simple diffusion (Parisi *et al*., 2007) [33]. And at the nick of time, the Agre group, while looking at Rhesus factor proteins in red cell membranes, noticed a consistent "contaminating" band on their gels of molecular weight at around 28 kD. They studied it further and concluded that this might be the water channels in red cell membranes. It was then isolated and purified, and antibodies showed that it was also expressed in cells of kidney proximal tubules and the thin descending limb of Henle - both of which are constitutively highly permeable to water. The defining moment came when the Agre group injected mRNA construct encoding this protein into an *in vitro* expression system, Xenopus oocytes. The oocyte membrane was normally very impermeable to water. But after injection of mRNA coding for the putative water channel, oocytes dropped into the distilled water did exactly that - they burst, while control oocytes remained intact. These findings made the Agre group realize that the mRNA injected had forced the oocytes to produce a new protein which, when moved to the oocyte membrane by cellular transport mechanisms, caused the membrane to become highly permeable to water. A series of subsequent papers confirmed that this new protein, called CHIP28 (channel-forming integral membrane protein of 28 kD), later renamed as AQP1 was the first water channel to be definitively identified (Preston *et al*., 1992) [35].

AQP1 had very little similarity to different notable proteins, except for the major integral protein (MIP) of lens fiber, which was subsequently renamed as AQP0. Soon thenceforth, additional AQP family members were identified from animal, yeast, bacterial and plant sources, with at least 13 AQPs in humans. In regard to their structure and functions, AQPs were simple proteins relative to their structure and functions compared to solute transporters and ion channels. AQPs represent a family of membrane channels that osmotically regulates water fluxes in numerous tissues and typically the transport of little solutes, as well as alcohol.

**AQP1**

The structure of aquaporin1 (AQP1) enumerated at 2.2 Å resolution consists of three topological elements, an extracellular and a cytoplasmic vestibule connected by an extended narrow pore or selectivity filter. Four bound waters were localized along three hydrophilic nodes within the selectivity filter, which punctuate an extremely hydrophobic pore segment. This combination of a long hydrophobic pore and a minimal number of solute binding sites facilitates rapid water transport through this molecule. Residues of the constriction region, in particular histidine182 was critical in establishing water specificity and remain conserved among all known water-specific channels. The analysis of the AQP1 pore also indicates that the transport of protons through this channel is highly energetically unfavourable (Sui *et al*., 2001) [44].

Taking into account the male reproductive tract the efferent ducts share an embryological origin with the renal proximal tubules where AQP1 is maximally expressed and 80% of the glomerular ultrafiltrate is absorbed (Schnermann *et al*., 1998) [40]. In the efferent ducts, this water channel was of great importance for regulating the concentration of testicular fluid, which requires rapid reabsorption (Clulow *et al*., 1998) [12]. Interestingly, studies in bulls evinced that localization of AQP1 disappeared in the basal membrane of epithelial cells of efferent ducts during the non-mating season, suggesting the presence of a different absorption pattern between mating and non-mating seasons with a higher water absorption in the mating one (Arrighi *et al*., 2016) [3].

The vas deferens in the male genitalia has the inherent ability to modify its luminal environment other than simply transit spermatozoa (Da Silva *et al*., 2006) [13]. In the desert, animals could acquire water from mobilized body storage during deprivatory conditions, which compensates for obligatory water losses (Takei *et al*., 2012) [46]. Camel also has an extraordinary capacity to survive without water supply. This is may be due to its ability to alter water homeostasis when under water deprivation. This functionality can be attributed to AQP1 which regulates the water balance to maintain an appropriate luminal environment in which sperm can continue their maturation.

Embryological studies show that AQP1 were expressed in murine syncytiotrophoblasts and in endothelium of placenta and chorion (Johnston *et al*., 2000) [23]. Moreover, AQP1 localization was also seen in the amniotic epithelium of both the chorionic plate and the fetal membranes. These observations suggest that AQPs in the amniotic epithelium facilitates water transport between the amniotic cavity and the fetal circulation (Mann *et al*., 2002) [28]. Further to study the effect of absence of this water channel on amniotic fluids volume and composition, AQP1 knockout mouse models were used. Indeed, with complete knockout of this water channel, the fetal mice showed increase in amniotic fluid volume and decreases in osmolality confirming that the AQP1 water channels were important in the regulation of amniotic fluid volume.

In rodents the head of the epididymis, including efferent ductules and the initial segment of the epididymis, has a major function in the reabsorption of luminal fluids (Clulow *et al*., 1998) [12]. This region expresses ERα receptors more abundantly than any other region in the tract and shows the greatest sensitivity to ERα disruptors (Hess, 2003) [18]. But the efferent ductules were distinctive in requiring each estrogen and androgen to mediate reabsorption of fluids, whereas the initial segment was dependent solely on androgen stimulation. Concurrent studies suggest that AQP1 was not regulated by androgens or estrogens in the male reproductive tract of rat but only are constitutively expressed. It appears that androgens have an inhibitory effect on AQP1 expression in these cells, but not in epithelial cells. Androgens could partially reverse this overexpression of AQP1 in the peritubular cells of efferent ductules. These parallel inconsistencies intimidates for further research in this field.

**AQP2**

The vas deferens plays a significant role in transepithelial water reabsorption of the reproductive tract and maintains the luminal environment during maturation of sperm. AQP2 localisation in the epithelial cells of the vas deferens was determined by a transgenic mouse approach to examine the mechanism of the principal cell-specific expression of AQP2 within the renal collecting duct. Its regulation in rat vas deferens is vasopressin independent unlike in renal tubules (Stevens *et al*., 2000) [42]. These channels were seen in some epithelial cells of the middle vas deferens and were mostly concentrated on the apical plasma membrane of principal cells in the distal vas deferens (ampulla), where AQP1 was also highly expressed. No expression was detected in the proximal region, and very little was observed on intracellular vesicles. The variable expression of AQP1 and AQP2 in different regions of the vas deferens may be due to the marked structural differences of principal cells along the vas deferens, reflective of diverse functional activities (Andonian and Hermo, 1999) [1].

Among domestic species, AQP2 are expressed in efferent ducts of adult dogs and in male genital tract of domestic cat (Arrighi & Aralla, 2014) [2]. This complexity of distribution patterns of AQPs in genital system suggests that both AQP1 and 2 might serve an important role in animal reproduction. The appearance of AQP1 in the camel vas deferens and no reaction of AQP2 thereby indicate that vas deferens may be less water permeable.

**AQP3**

As a member of the aquaglyceroporin family it allows the transport of glycerol along with water. Its function can also be attributed to the transport of water and cryoprotectants (CPAs) across cell membranes thereby preventing osmotic damage (Kumar *et al*., 2015) [24]. Experiments have shown that AQP3s permeability to glycerol and other CPAs was positively correlated with motility and viability of frozen thawed boar spermatozoa evaluated at 30 and 240 minutes post thawing, making it a freezability marker for boar spermatozoa (Prieto Martinez *et al.*, 2017) [36].

This is the first identified sperm protein responsible for efficient post-copulatory sperm osmoadaptation, thereby influencing male fertility. Mammalian sperm can reduce the negative impact of hypotonic cell swelling by means of

regulatory volume decrease (RVD), which involves efficient volume regulation driven by active solute transport and rapid transmembrane water movement. Simple porosity theories contemplate aquaporin as inert pores that increase the diffusion permeability of plasma membranes. RVD begins with a hypotonic stress-induced water influx, followed by active solute transport that enables osmolyte efflux and provides the driving force for water to exit. AQP3 functions as osmosensing/mechanosensing system for the initial sensing of cell swelling and "trigger" succeeding RVD events such as solute transport and bodily structure reconstruction. Thus it was concluded that AQP3 is a key fluid regulator essential for sperm regulatory volume decrease (RVD) upon hypotonicity-induced cell swelling. When sperm utilizes post-copulatory hypotonicity for motility activation, AQP3 serves to protect sperm from swelling induced mechanical membrane stretch, thus optimizing the "trade-off" between sperm motility and cell swelling upon physiological hypotonicity (Chen *et al*., 2011) [11].

**AQP4**

AQP is the predominant water channel protein in mammalian brains and its distribution was highest in the perivascular and subpial astrocyte end-feet. It is a critical component of an integrated water and potassium homeostasis and is implicated in several neurological conditions such as edema and seizures. The water permeability via AQP4 around BBB (Blood Brain Barrier) is of significance because brain edema develops on breach in the BBB. Animal models of AQP4 null mice were protected from cytotoxic brain edema, including hyponatremia and early focal cerebral ischemia (Papadopoulos and Verkman, 2007) [32]. On the other hand, AQP4 null mice were more sensitive for vasogenic edema due to increased permeability of capillary endothelial cells associated with brain tumors. AQP4 may also be involved in limiting the entry of edematous fluid from the tumor bed into the brain parenchyma. In astrocytes around the vicinity of edematous brain tumor, AQP4 expression were strongly up-regulated and was not polarized to just the end-feet, but was also seen throughout the entire cell membranes in immunohistochemical studies of human brain tumors (Badaut *et al*., 2003) [4]. Experimental studies in AQP4 null mice revealed prolonged seizure and delayed potassium reuptake from the extracellular space during cortical spreading depression. These series of concurrent findings indicate that AQP4 plays a key role in these pathological conditions. The ability of AQP molecule to modulate water fluxes in mice models has provided new insights into brain-water homeostasis and suggested a number of new research directions. These advances guide us towards the development of AQP inhibitors and activators to establish the benefit modulating the function of these water channels.

In mammals, the hypothalamus-pituitary-ovary axis (HPOA) is a key element that regulates the reproductive system. A pulsatile GnRH signal is required for secretion of the gonadotropin LH and FSH from the pituitary, which drives steroidogenesis and follicular development (Belchetz *et al*., 1978) [5]. During these processes, hormonal signaling is integrated by the glial cells. Astrocytes can release factors that modulate the activity of GnRH neurons, such as prostaglandin E2 (PGE2), transforming growth factor a, b1, b2 (TGFa, TGFb1, TGFb2), basic fibroblast growth factor (bFGF) and insulin-like growth factor 1 (IGF-1). In addition, glial cells metabolize hormones as well as synthesize active metabolites that affect neuronal function. Astrocytes mediate estrogen-induced synaptic plasticity of GnRH-releasing neurons in hypothalamic areas. Astrocytes also regulate local intraneuronal production of steroids, which can intervene in feedback control of GnRH neurons as negative or positive signals. AQP4 knockout induced astrocytic dysfunction and sex specific alterations of neurotransmission and decreased ovarian hormones (Sun *et al*., 2007) [45]. Estradiol and progesterone levels will be significantly lower so fertility tests induced a lower rate of pregnancy and decreased litter size. Therefore, it can be concluded that AQP4 deficiency subsequently results in reproductive disorder due to astrocytic dysfunction.

**AQP5**

AQP5 was localised in the vaginal epithelium, apical cell layers of cervix, uterus, oviduct, granulosa cells and also in embryos where it facilitates vaginal lubrication, cervical water balance during pregnancy and parturition, water homeostasis of uterus and oviduct, gamete transport, fertilization, early embryonic development and implantation in the female reproductive tract. Immunohistochemical studies show its presence in the testes, epididymis, vas deferens in the male reproductive tract, associated with movement of water and small uncharged solute molecule at critical sites (Zhang *et al*., 2012) [52].

Oxytocin selectively influences the expression of AQP5 at the end of pregnancy and may serve as a marker that indicates the initiation of delivery in rats (Ducza *et al*., 2014) [15]. The uterine expression of mRNAs and proteins for AQP5 in pregnant gilts during placentation and effect of factors such as Progesterone, Estrogen, Arachidonic acid, Oxytocin, cAMP and Forskolin on their expression was studied. Estradiol has been found to stimulate AQP5 mRNA expression, but progesterone predominantly inhibited AQP5 mRNA levels. In turn, at the protein level, steroid hormones and arachidonic acid stimulated the expression of both AQP1 and 5, but cAMP and Forskolin stimulated only AQP5 (Skowronska *et al*., 2016) [41].

**AQP6**

AQP6 was exclusively localized in intracellular vesicles containing $H^+$-ATPase but was absent in the plasma membrane (Yasui *et al.*, 1999) [50]. AQP6 expression in mammalian epithelial cells by transient transfection with rat AQP6 cDNA construct revealed that the protein remained in intracellular sites. The addition of the GFP (Green Fluorescence Protein) tag to the N terminus of the structure, but not the C terminus, causes AQP6 to traffic to the plasma membrane. This suggests that not C terminus but N terminus of AQP6 is important for the restriction of AQP6 to intracellular sites. The underlying mechanism was that GFP interferes with the recognition of the signal in the N-terminal which determines AQP6 trafficking. Some literature suggested that AQP6 co-localizes with $H^+$- ATPase in intracellular vesicles of acid-secreting type-A intercalated cells where pH drop was usually 5.0 or lower. Thus, AQP6 can act as an acid-induced anion channel in type-A intercalated cells in collecting duct and needs further studies to throw light into inconsistencies. Numerous experiments also indicated that AQP6 has an anion permeability sequence of Nitrate » Iodide » Bromide » Chloride » Sulphate. This order resembles that of the GABA and glycine-gated channels since firstly AQP6 has a single ion-binding pore, GABA or glycine-gated channels have been proposed to contain a multi-ion-binding pore. Second, high permeability for nitrate was

observed even at an alkaline pH of about 7.4. This implies either that the channel was already open to some extent even under basal conditions or permeation by nitrate induces channel gating of pore-lining residue Thr-63 in AQP6 for nitrate selectivity. The high permeability to nitrate and the single ion-binding pore for AQP6 can also be explained by structural assessment i.e. a single ion- binding pore in each subunit with monotonic mole fraction behavior with $NO_3$/Cl mixture. A few unique amino acid residues such as Tyr-34 and Thr-63(residues which are critical for water channel function) are very well conserved in AQP6, suggesting that very subtle differences can lead to major disparity in biophysical function (Ikeda *et al*., 2002) [20].

**AQP7**

AQP7 belongs to aquaglyceroporin family and sequential studies of this protein revealed a common additional conserved Asp residue near the second NPA box that enlarges the pore to permit glycerol (Ishibashi *et al*., 2011) [21]. AQP7 was the first glycerol channel identified in the adipose tissue of humans and rodents and its expression was confined to adipocytes in the membranes surrounding the lipid droplets, which migrated to the plasma membrane in response to β-adrenergic stimuli. Other studies found its localization in the endothelial cells of small vessels within the adipose tissue (Lebeck *et al*., 2012) [27]. First evidence for the fundamental role of AQP7 was demonstrated to be the release of glycerol from adipocytes. AQP7 functions as a glycerol channel which was regulated negatively by insulin, positively by fasting and in mice models were more abundantly expressed in obese mice with insulin resistance. Epinephrine, glucagon and ACTH were ineffective at regulating AQP7 mRNA expression in animal models. However, epinephrine induced AQP7 translocation to the plasma membranes from the intracellular regions which led into the conclusion that insulin coordinates AQP7 regulation in adipose tissues in fasting/refeeding conditions (Kuriyama *et al*., 2002) [25].

**AQP8**

Rat hepatocytes treated with cAMP agonists exhibited 2 fold increase in water permeability by relocalization of intracellular AQP8 to the plasma membrane and a six fold increase in water permeability of canalicular plasma membrane domains. Studies using AQP8 knockout mice as control showed AQP8 immunolocalization in the plasma membranes of hepatocytes in mice but with weak intracellular labeling. Through these studies it can be concluded that cAMP-regulates AQP8 water permeability. Localisation of this protein was also seen in testicular and epididymal spermatozoa in rats, whereas the presence of AQP8 on murine spermatozoa was confirmed with Western blotting. It has also been reported in the cytoplasmic droplet of epididymal spermatozoa. When Regulatory Volume Decrease (RVD) activity of epididymal spermatozoa is inhibited by quinine, cell swelling can be detected by volume measurement and an angulation of the sperm tail at the site of the cytoplasmic droplet. Therefore, AQP8 may serve as water channels in the physiological RVD that spermatozoa have to undergo in the female tract. On the other hand, if there is no other alternative water channel when AQP8 is nullified, the sperm would not experience fast swelling upon encountering the hypo-osmotic challenge in the female tract in the first place, hence no demand for effective RVD. These findings explain why the AQP8 null male mice are still fertile (Yeung *et al*., 2009) [51]. A definitive role needs to be confirmed by future experiments examining water transport in spermatozoa from these transgenic animals.

**AQP9**

AQP9 was described as promiscuous AQP that allows passage of not only water but also other solutes such as urea and polyols including mannitol, purines and pyrimidines in the male reproductive tract. AQP9 was enriched on the apical (but not in basolateral) membrane of non-ciliated cells in the efferent duct and principal cells of the epididymis (rat and human) and vas deferens, where plays a key role in fluid reabsorption. AQP9 may have functions in addition to fluid transport across membranes. This may explain the presence of more than one member of the AQP family in the same membrane domain of some epithelial cells lining different portions of the male reproductive tract. Non-ciliated cells in the efferent ducts express AQP1 which were confined on the apical and basolateral plasma membranes; in contrast AQP9 expression was mostly seen in non-ciliated cells in the apical but not in the basolateral domains. 50%-80% of the fluid reabsorption happening in the efferent ducts was attributed to AQP. Fluid reabsorption results in concentration of sperm in this segment. So, expressions in such cells require exposure to appropriate levels of estrogen. The presence of AQP9 in the efferent ducts might compensate partially for the loss of AQP1 from the tubular tissues. Permeability studies on perfused, isolated efferent ducts will be necessary to determine the relative contribution of AQP1 and AQP9 to transepithelial fluid movement. Putative targeting signals on a given protein can be interpreted in a cell-type specific fashion. Studies on colchicine-treated rat models strongly suggest that in the epididymis AQP9 follows a constitutive pathway of plasma membrane insertion and it does not rapidly recycle between the plasma membrane and intracellular vesicles. Microtubule disruption by colchicine (or by cold exposure of tissue) resulted in a marked redistribution of rapidly recycling membrane proteins from the cell surface into intracellular vesicles whereas non-recycling or slowly recycling proteins are not affected by this drive. AQP9 clearly falls into the latter category as its distribution was similar in colchicine treated and control rat's redistribution of rapidly recycling membrane proteins from the cell surface into intracellular vesicles whereas non-recycling or slowly recycling proteins are not affected by this maneuver (Breton *et al.,* 1998) [7]. A progressive increase in AQP9 expression in the epididymis was seen during postnatal development, both in terms of the number of tubule segments expressing AQP9 and the intensity of AQP9 staining in individual cells within these segments. In 3- to 4-week postnatal rats, the expression levels and AQP9 distribution were indistinguishable from the adult pattern. Because androgens are present throughout the perinatal period and their levels increase during puberty warranting that AQP9 expression is modulated by androgens. The heterogenous and segment-specific expression of AQP9 and other AQPs along the male reproductive tract suggests that fluid reabsorption and secretion in these tissues could be locally modulated by physiological regulation of AQP expression and/or function.

**AQP10**

AQP10 was grouped under aquaglyceroporins which are associated with water and small solute absorption and secretion in the gastrointestinal tract, regulation of glycerol metabolism in the adipose tissues, water balance maintenance in the male reproductive tract and also forms a barrier in the skin for defense related mechanisms. All aquaglyceroporin

protein sequencing revealed a common additional conserved Asp residue near the second NPA box that enlarges the pore to permit glycerol. AQP10 protein is expressed in humans, while in mice and in other animal species it exist as a non-functional pseudogene. Its presence was seen in both adipocyte and capillary plasma membranes of human adipose tissue. The expression of this protein in the adipose tissue is particularly important for the maintenance of normal or low glycerol contents inside the adipocyte, thus protecting humans from obesity (Laforenza *et al*., 2016) [26]. Glycerol fluxes across plasma membranes of adipocytes (and likely enterocytes in the duodenum, where reported pH was acidic) were stimulated by low pH and undisputedly linked to hAQP10, a protein which is highly physiologically relevant for glycerol flow in these cell types. pH regulation in such tissues were achieved by a cytoplasmic, glycerol-specific gate and likely, a widened ar/R filter, both unique to hAQP10, correlating with intracellular acidification of adipocytes observed during lipolysis (Gotfryd *et al*., 2018) [17].

**AQP11**

AQP11 also comes under superaquaporin family and differs from the others not only in their unusual Asn-Pro-Ala (NPA) motifs, where the first of the two highly conserved hydrophobic NPA motif forms the water pore in the prototypical AQPs, but this family contains cysteine instead of an alanine and their localization is intracellular. AQP11 localization in sperm was seen in the caudal cytoplasm of the elongating spermatid. As spermiation progresses, localization differs and intensity of AQP11 staining paralleled the fate of the residual cytoplasm during the formation and elimination of the residual bodies. At the start of nuclear condensation and tail formation process, the cytoplasm of the spermatid moved caudally and no AQP11 could be detected in some studies. Only when development of the spermatid reached around stage 16, AQP11 was first expressed. The stronger expression of this protein was found to be similar with the contraction of the caudal cytoplasm, which gradually became invaginated by the Sertoli cell while it moved anterior to the sperm head. As this cytoplasm is secured by the Sertoli cell processes, the elongating spermatids migrate towards the lumen until they detach from the seminiferous epithelium, leaving behind their bulk cytoplasm which forms the residual bodies that are phagocytosed and gradually digested by the Sertoli cells. Thus, AQP11 was suggested as a marker for the process of spermiation. In the testes, the process of spermiogenesis and spermiation produces a large redundant cytoplasmic volume and components which confer a heavy burden on the Sertoli cell to maintain the germinal epithelium homeostasis. AQP11 may be important in facilitating the elimination of these surplus intracellular organelles and their contents without causing much damage to the Sertoli cells after their phagocytosis and degradation. This would allow not only recycling of surplus proteins and organelles of spermatid origin, but also homeostasis of the Sertoli cells in the support of spermatogenesis, which was demonstrated by the clearance of apoptotic spermatogenic cells, the failure of which may result in decreased sperm production. Malfunction of this clearance mechanism may even cause a breakdown of self tolerance leading to autoimmune orchitis. On the other hand, it has been demonstrated most recently that ATP can be generated by the sertoli cells after phagocytosis from apoptotic spermatogenic cells and the residual bodies (Xiong *et al*., 2009) [49].

**AQP12**

AQP12 has been reported in mammalian species and localization as of today is seen only in the pancreatic acinar cells (Buffoli, 2010) [8]. The specific location has not been established for this subfamily, but its presence in the pancreatic acinar cells implies a possible pathway in digestive enzyme secretion (Rojek *et al*., 2008) [38]. AQP12 has a higher identity with AQP11 (32%) compared with other AQPs, which show less than 15% identity. A structural feature of AQP12 is the lack of an N-terminal cytoplasmic region, in contrast to all other known aquaporins (Ishibashi, 2009) [22]. The pancreatic acinar cells synthesizes the digestive enzymes which are then stored in secretory vesicles called the zymogen granules in the apical pole of the cell. The immature secretory granules called the condensing vacuoles containing the secretory proteins in a dilute form bud off the Golgi after the enzymes are synthesized in the endoplasmic reticulum. Then, the contents get concentrated when the condensing vacuoles undergo a packaging process. The granules progressively reduce their volume and become mature zymogen granules. Elevated intracellular $Ca2^{+}$ stimulates the acinar cells to secrete digestive enzymes resulting in the fusion of zymogen granules with the plasma membrane and concurrent exocytosis. Zymogen granules possess $Cl^{-}$ and ATP-sensitive $K^{+}$ selective ion channels at the membrane and GTP provokes rapid swelling of isolated zymogen granules confirming the presence of AQPs in the membrane of intracellular zymogen granules in acinar cells.

**Involvement of AQP in bowel disorders**

Gut involvement of AQPs was firm in several mechanisms that mediate water transport (intestinal permeableness and fluid secretion/absorption). Regarding AQPs regulation, it was known that substances such as hormones and dietary components act by modifying their expression and altering fluid homeostasis in the gut. Inflammatory bowel diseases (IBDs) are inflammatory relapsing diseases of gastrointestinal tract with a chronic aberrant stimulation of immune system, gut inflammation and leakage of fluid, solutes, and lipids in bowel mucosa. IBD is also characterized by dysregulation in electrolyte and water transport with resultant alteration of permeability resulting diarrhea. The remarkable increase of intestinal membrane permeability observed in these diseases has suggested the participation of AQPs along with cytokines(TNF and IL-1b) which acts as an important signaling molecules in the intestinal immune system and can also be correlated to the severity of inflammation (Strober and Fuss, 2011) [43]. A direct correlation was derived between AQPs and diarrhea, defining AQP contribution to diarrhea caused by attaching and effacing bacteria (i.e., enterohemorrhagic *E. coli* and enteropathogenic *E. coli*) pathogenesis. A possible relationship between AQPs (AQP1, AQP3 and AQP8) expression and NF-Kb (nuclear factor kappa-light chain-enhancer of activatred B cells) pathway in a model of IBD has been established. Findings indicate NF-kB pathway as the main regulator of several processes (pro-inflammatory cytokine production, leukocyte recruitment, or cell survival), and its involvement in relation to AQPs. Regarding the possible link between TNFa and AQPs, it has been evidenced that TNFa acts by modulating AQP3 expression (down- or up-regulation) according to the cell type involved, through different signaling pathways (Horie *et al*., 2009) [19]. Recent studies suggest that AQP3 downregulation is mediated by the inhibition of constitutive transcriptional activity at the AQP3 promoter in HT- 29 cells (Peplowski *et*

*al*., 2017) [34]. Another cell line (CMT93) demonstrated that IFNg limits epithelial AQP1 expression through the activation JAK/STAT3 pathway (Dicay *et al*., 2015) [14]. The potential role of AQPs in both cases of severe IBDs (Crohn's disease and ulcerative colitis) has been demonstrated and different distribution patterns of these channel proteins in the gut and the existence of a direct relationship between intestine inflammation and physiological water/solute trafficking and regulation has also been established.

**AQP involvement in bone and cartilage diseases**

AQP involvement has been found in several inflammatory diseases affecting bone and cartilage. In rheumatoid arthritis (RA) elevated levels of inflammatory cytokines secreted by activated B and T cells causes damage to the cartilage and bone. Different AQP localization has also been found in cartilagenous cells where they regulate the trafficking of ions and molecules and thus regulate the cartilage physiology. In particular, it was evidenced that in synovial tissues from patients with osteoarthritis (OA) and RA, TNFa regulates either AQP9 mRNA and protein expression (Nagahara *et al*., 2005) [31]. So, cytokines can alter the activity of glucose transporters which is important for chondrocyte metabolism thus influencing AQP function (Richardson *et al*., 2003) [37]. According to this mechanism, AQP1 present on chondrocyte membrane could act as regulator of metabolic or extracellular water matrix suggesting that chondrocytes might respond to changes in their ionic and osmotic environment modifying volume regulatory behavior (Trujillo *et al*., 2004) [47]. The direct involvement of AQPs in the pathogenesis associated with this disease was investigated in a model of OA cartilage where AQP1 mRNA was evaluated by RT-PCR and it was evinced that up-regulation of AQP1 was related to the chondrocyte apoptosis (Gao *et al*., 2011) [16]. AQP4 accountability in pathogenesis of RA was demonstrated in Adjuvant-Induced-Arthritis (AIA) rat model. Results demonstrated that Acetazolamide treatment was responsible for reduced mRNA levels of collagen type II and aggrecan in the cultured AIA chondrocytes. Acetazolamide induced AQP4 inhibition promoted extra cellular matrix production of AIA chondrocytes *in vitro* (Cai *et al*., 2017) [9].

**Inflammatory diseases in domestic animal species: the involvement of aquaporins**

In the recent years, inflammatory diseases in domestic animal species has been studied but the available data are limited considering several limitations for ethical problem in these species with respect to laboratory animals. However, there are evidences suggesting that animals as well as humans can suffer from several inflammatory diseases whose possible mechanisms are not always well-defined. Although, different diseases have been investigated in domestic species, fewer have been studied to draw the possible link between AQPs and inflammation. The most investigated species is the dog most likely because of its similarity to humans. Inflammation-based diseases in different organs and systems like the gut, central nervous system, and lung have been investigated in dog species with the perspective to clarify their pathophysiology. Studies were performed on various aspects regarding dysbiosis networks in dog IBDs, evidencing differences and similarities with humans (Cerquetella *et al*., 2010) [10]. The results of this study provides new important contributes for translational medicine requiring further development of scientific research for understanding differences between dog and human in some bacterial species. Studies showed an increase of AQP4 and IL6 levels in cerebrospinal fluid (CSF) of dogs affected by idiopathic communicating internal hydrocephalus and a reduction of these proteins after ventriculoperitoneal shunting (Schmidt *et al*., 2016) [39]. Also studies on acute respiratory distress syndrome in beagle dogs showed a clear inflammatory process characterized by TNFa increase which facilitates the secretion of cytokines, such as IL-1A, IL-6, and IL-10 (Zhao *et al*., 2012) [53]. Moreover, the decreased AQP1 and AQP5 expression which was observed as possible consequence of pulmonary capillary membrane barrier damage suggests that AQPs might have possible involvement in the regulation of fluid trafficking mechanisms along this membrane. Findings in avian species relates to the expression of AQPs at level of nasal glands influencing its fluid secretion. AQP1 and AQP5 play a role in modulating nasal fluid secretion and renders it hypertonic, differently from vertebrates (Müller *et al*., 2006) [30].

**Conclusion**

Aquaporins are present in almost all cells, tissues and organs which plays a vital role in transport of water and solutes across the lipid bilayer. AQP1 and AQP9 has been mostly explored and found to be involved in the dynamics of secretion/reabsorption along the efferent ducts, epididymis and vas deferens. The underlying mechanism is still on debate and its localization in the non-ciliated cells of the efferent ducts has led some authors suggest that these proteins are crucial for the transcellular route that compliments fluid phase endocytosis. Most aquaglyceroporins and superaquaporins has been localized in the sperm facilitating glycerol transport evincing as a potential cryotolerance marker in boar and bull spermatozoa. AQPs has also been associated with several bodily dysfunction making it as a possible candidate for therapeutic potential target in modulating inflammatory cytokines, cell migration and mediator release. Since, AQPs are ubiquitous in nature it warrants further researches to unravel the signaling pathways involved in their regulation and function.

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