Lipid entry of small organic and inorganic molecules.

Lipid bilayer in cell separates the intracellular and extracellular cytoplasmic matrix, maintaining the structural integrity of the cell. It plays a vital role in exchange of substances between the cells and its environment, providing energy for the physiological processes. The major component of all living organisms including all vertebrates, invertebrates, unicellular organisms and plants is water.

Water freely diffuses the lipid membrane only at a limited rate. Movement of these water molecules across the membranes is essential for life. It was presumed that transport of water molecules is via simple diffusion through the lipid bilayer until the discovery of aquaporins. Earlier in 1970’s prediction towards the presence of water channel in cell membranes were described. It was Peter Agre and colleagues who named water channel as aquaporins (AQPs)(1) (Preston et al., 1992). Aquaporins exist as different isoforms till date there are 13 isoforms (AQP0-12) identified in mammals.

Aquaporins are family of membrane intrinsic proteins, forming pores facilitating massive transport of water across the membranes. AQPs based on the sequence analysis has been classified under three subtypes classical, aquaglyceroporins and orthodox aquaporins (2) (3) (4) (Agre and Kozono, 2003; Zardoya, 2005; Nozaki et al., 2008). Classical AQPs 1, 2, 4, 5, 6&8 are selective water channels and restricts the entry of small organic and inorganic molecules.

Aquaglyceroporins 3, 7, 9 & 10 are non-selective channels permeable to water, urea, glycerol and small non-electrolytes. Unorthodox aquaporins are AQPs 11&12 are being investigated. In addition, AQPs are also facilitate

transport of gases like carbon dioxide (5) (6) (7) (Nakhoul et al., 1998; Cooper and Boron, 1998; Uehlein et al., 2003), nitric oxide (8) (Herrera and Garvin, 2007), and ammonia (9) (10) (11) (Holm et al.

, 2005; Musa-Aziz et al., 2009; Gruswitz et al., 2010).

Although some gases are theoretically small enough to pass through the aqueous pore of the AQP, compelling evidence is lacking for physiologically relevant gas transport, partly because the intrinsic lipid-mediated membrane permeability to most gases is high (12) (13) (14) (Yang et al., 2006; Missner et al., 2008; Madeira et al., 2016). Current evidence suggests that most, if not all, significant biological functions of the mammalian AQPs, including those described here, can be attributed to AQP-facilitated water and/or glycerol transport. Structure of AquaporinsAquaporins are simple proteins concerning the structure and functions compared to ion channels.

Several mammalian AQPs have been determined using X-ray structures each with ~30-kDa monomer, spanning six helical domains with cytoplasmic oriented amino and carboxy termini, including NPA sequences in their short helical segments forming a distinct water pore. In addition to the effect of NPA motifs, reentrant loops also help to maintain the configuration of the bipolar water file, the two-helices generate two electrostatic dipoles which point toward the center of the channel, forcing water dipoles to orient in the opposite direction. Under their influence, water molecules tend to point their oxygens toward the center of the channel. The movement of water occurs as a single file through the pore via electrostatic and steric factors (15) (16)

(Hub et al., 2008; Khalali-Araghi et al., 2013). AQP1, AQP2, AQP4, AQP5 and AQP8 primarily functions as bidirectional water-selective transporters.

It is well established that heavy metals can directly interact with AQPs thereby affecting their activity. Studies have shown that heavy metals are found to have inhibitory effect on aquaporins, especially mercury (17) (Hasegawa et al., 1994). Copper and nickel involving amino acids in loop C and E mediates AQP inhibition (18) (19) (Zelenina et al., 2003, 2004). Further silver and gold is also reported to have inhibitory effect on AQPs (20) (Niemietz and Tyerman, 2002). The new insights into the regulation and functions of AQPs in reproduction are revealed in recent years. This review extensively discusses the distribution of AQPs in male reproductive tissues and their functions, and, mainly focuses on the recent advances in our understanding of the physiological and pathophysiological roles of AQPs in male reproductive systems.

Functions of Aquaporins in Male Reproductive SystemFluid secretion and reabsorption are of central importance in male reproductive (MR) physiology (21) (Calamita, 2001). Water movement across the male reproductive tract plays a pivotal role in maintaining the luminal environment for spermatogenesis and also in increasing the concentration of sperm. The water channel aquaporins is found to play an important role in reproductive system facilitating transepithelial fluid secretion in exocrine glands and other secretory epithelia (22) (Tradtrantip et al.

, 2009). Multiple aquaporins are found to be expressed in the male reproductive system and few have been reported to be tissue specific and

further regulated by steroid hormones. In regard, to human male reproductive system there are only few reports on aquaporins (23) (24) (Zaniboni and Bakst, 2004; Zaniboni et al., 2004).

Moreover, the specific expression pattern of AQPs suggests transport of water is locally modulated. Alteration in the expression, function and/or regulation of AQPs lead to disorders of male reproductive system. AQPs aids in transcellular transport, maintaining water homeostasis and also is strongly associated with male reproductive system. Aquaporins Role in SteroidogenesisSteroidogenesis, the process by which biologically active steroid hormones being synthesized from cholesterol, which is of prime importance for normal reproductive function and bodily homeostasis.

Testicular steroidogenesis produces testosterone required for fertility being synthesized by Leydig cells. AQP9 prominently being present in the plasma and intracellular membrane of interstitial Leydig cells (25) (26) (Elkjaer et al., 2000; Badran and Hermo, 2002). Hermo, 2004 has also reported the presence of AQP0 in leydig cells (25) . Sex steroids by either dependent or independent pathways regulates aquaporins 1 and 9 in leydig cells (27) (Oliveira et al., 2005). Recently it has been reported that, silencing testicular AQP11 is found to regulate steroidogenic genes in syrian hamsters indicating its role in fertility (28) (Shannonhouse et al., 2014).

However, many of the underlying molecular mechanisms of aquaporins in testicular steroidogenesis remain elusive. These interesting findings that, aquaporins being regulated by steroid hormones opens up the arena for future research to investigate the role of aquaporins in testicular

steroidogenesis leading to male infertility. Aquaporins Role in SpermatogenesisFluid secretion and absorption in male reproductive tract is necessary for spermatogenesis, including the maturation of spermatozoa, maintaining concentration and storage in seminiferous vessels (29) (Yeung et al., 2009). Fluid movement across the testis indicates the presence of multiple aquaporins.

Functional and/or regulation changes in the male reproductive system leads to sub-fertility/Infertility. The physiological significance and the underlying mechanisms in male reproductive function is poorly understood. In late 90’s it was reported that AQP1 (30) (31) (Liu et al., 1995, Lu et al., 2008) was not detected in spermatozoa and AQP2 (32)(Fushimi et al.

, 1993), AQP3 and AQP4 (Frigeri et al., 1995) were all absent in testis (33). Later in teleosts, AQP1 has been expressed in germ cells especially in spermatozoa and spermatids indicating its role in spermatogenesis (34) (35) (36) (Zilli et al., 2011, Guo et al.

, 2017; Cedra et al., 2017). AQP7 and AQP8 present in ejaculated human spermatozoa found to play an important role in male fertility regulating sperm volume (29)(Yeung et al., 2009). The presence of AQP7 in the spermatids, as well as in epididymal spermatozoa, suggests its role in late spermatogenesis (37) (Sohara et al., 2009). It has been reported that AQP7 plays an important role in human sperm motility (38) (Moretti et al.

, 2012). AQP 7 and AQP8 are not uniformly distributed throughout the testis, AQP7 mRNA being expressed in late spermatogenesis and protein localization varies depending on the stages of spermatogenesis (21)

(Calamita et al., 2001a, b). AQP8 mRNA being expressed uniformly in all seminiferous tubules and protein was found in the germ cells intracellularly and throughout the plasma membrane (39) (Calamita et al., 2001b). AQP3 and AQP8 helps in maintenance of sperm motility, sperm tolerance and fertilization capacity(40) (Zhang et al., 2018).

AQP11 aids in recycling of surplus cytoplasmic components of elongated plasmids and maintains Sertoli cells supporting spermatogenesis (41) (Yeung and Cooper., 2010). Further studies on AQPs in the developing rat testis in accordance with the maturation of germ cells support the roles of AQPs in spermatogenesis (39) (42) (Calamita et al., 2001a; Kageyama et al.

, 2001). Differential expression of these AQP 7 and AQP8 in adult and developing rat germ cells signifies its role in spermatogenesis. AQP7 conferred protection against sperm volume shift and impaired functions (43) (Kasimanickam et al., 2017). Sperm volume regulation and ROS elimination is contained by AQPs in humans indicating its role in normal functioning of spermatogenesis(44) (Laforenza et al.

, 2017). AQP1 reduction in mice showed significant dilation, and accumulation of spermatozoa in the rete testis and efferent ducts (45) (Danielian et al., 2016). Even most of the domestic animals do express AQPs in sperm and spermatozoa indicating its role in spermatogenesis and fluid homeostasis (46) (47) (48)(Arrighi & Aralla, 2014; Arrighi et al., 2016; Schimming et al., 2017). It has been shown that AQP7 was altered and not AQP9, in the freezing medium of boar sperm head after freeze thawing (49) (Vicente- Carrillo et al., 2016).

The boar and bull spermatozoa with higher AQP3 and AQP7 expression is resistant to cryopreservation procedures, which includes the osmotic shock of freezing media(50) (51) (Prieto- Martínez, Morató et al., 2016; Prieto-Martínez et al., 2017). AQP3, AQP7 and AQP11 role in maintaining the sperm freeze thawing procedures has been extensively reviewed (52) (Yeste et al., 2017). Sertoli cells in spermatogenic epithelium serves as the vehicle for transport of sperm from testis to epididymis and AQP0 has been reported to be expressed in a semicircular pattern (25)(Hermo et al., 2004).

Transgenic mice were used to study the physiological changes of AQPs in fluid absorption and it was reported that AQP8 deficient mice had subtle physiological differences (53)(Yang et al., 2005). In AQP8 null mice, the size of testis was increased with higher ratio of spermatogenic cells to Sertoli cells with reduced water permeability indicating the possibility of abnormal sperm.

AQP9 was intensely expressed in microvilli of non-ciliated cells (54) (26) (27) (Pastor-Soler et al., 2001; Badran and Hermo, 2002; Oliveira et al., 2005). AQP9 was abundantly expressed on the apical membrane of principal cells along the entire epididymis in O. nigripes (55) (Tatiana Prata Menezes et al.

, 2017). Human epididymis too incurs the similar pattern of AQP9 expression enabling rapid cellular movement the requirement for growing spermatocytes as found in rats(54) (Pastor-Soler et al., 2001). AQP-9 variation in varicocele testes, leads to hypospermatogenesis (56)(Arena et al., 2011). AQPs physiological functions in germ cell development and sperm motility molecular mechanisms remains elusive (57)(Boj et al., 2015).

Furthermore, the expression is variable in different stages during spermatogenesis already been demonstrated to be at the basis of some forms of male sub-fertility and infertility. Aquaporins maintains extracellular osmolality during the process of cell volume regulation in the distal region of the hamster epididymis (58) (Cooper & Yeung, 2003). About 50 to 90% of luminal fluid secreted by seminiferous tubules is reabsorbed in the efferent ductules (59) (Clulow et al., 1998). This rapid absorption occurs vis ion transporters Na+/H+ exchanger-3 (60) (61) (Lee et al., 2001; Leung et al.

, 2001), Cl–/ HCO3 – exchanger (60) (Lee et al., 2001) and chloride channel cystic fibrosis transmembrane regulator (CFTR) (60) (61)(Lee et al., 2001; Leung et al., 2001) and specific expression of AQPs indicates its role in transepithelial movement. Intense expression of AQP1 in the brush border and the cilia of ciliated cells of efferent ductules (26) (27) (31) (Badran and Hermo, 2002; Oliveira et al., 2005, Lu et al.

, 2008), (62)on the other hand AQP1 null mice, impairment wasn’t observed in the efferent ductules (62)(Zhou et al., 2001) indicating the presence of other aquaporins. Moreover, AQP1 is absent from the epithelium of the epididymis, but it was expressed over the endothelial cells of the vascular channels of epididymis(26) (27) (Badran and Hermo, 2002; Oliveira et al., 2005). The expression of AQP1 and AQP9 in the efferent ductules might be regulated by estrogen because the expression of these two isoforms of AQPs was significantly reduced in the efferent ductules of mice deficient in ER? (27) (63)(Oliveira et al., 2005; Ruz et al.

, 2006). Androgen hormone is also found to regulate AQP1 and AQP9 in the epididymis under ethanol consumption indicating its role in efferent ductules and AQP10 over the epithelium in efferent ductules (25) (Hermo et al., 2004). In rats, AQP9 strongly expressed in principal cells rather than the basal cells of the epididymis (26) (27) (64) (Badran and Hermo, 2002; Oliveira et al., 2005; Da Silva et al.

, 2006). AQP9 also being expressed in human epididymis is important to prevent luminal dehydration(63, 65) (Cheung et al., 2003; Ruz et al.

, 2006). Other AQPs mRNA, AQP2, AQP5, AQP7 and AQP11 were detected in the epithelium of epididymis (64)(Da Silva et al., 2006).

AQPs are present in the principal and basal cells of epididymis and plays an important role in fluid homeostasis of male reproductive tract (66)(Castro et al., 2017). It has been reported that AQP5 along with AQP9 is co-localized in the apical membrane of corpus and cauda regions (67, 68) (Pastor-Soler et al., 2010; Hermo and Smith, 2011). Interestingly AQP2 is present in distal region of epidydimis throughout the developmental stage and in the principal cells of the corpus and caudal (69, 70)(Arrighi et al., 2010a, b).

Exclusively in the basal cells of epididymis AQP3 being expressed (25) (Hermo et al., 2004). Further AQP8 expression neither gene or protein expression was observed (39, 64)(Calamita et al., 2001b; Da Silva et al., 2006), in contrast AQP8 expression in basal cells was reported(71) (Elkjaer et al.

, 2001). AQP8 and 9 is cell-specific in testis in the efferent ducts and epididymis, expression of AQP1 and 9 is cell, region, and tissue specific, and, does not appear to be regulated by androgens (26) (Badran and Hermo, 2002). AQP9 expression was barely detectable in microvilli of principal cells and under underfed condition was completely absent in epididymis and cauda regions (69, 70) (Arrighi et al., 2010a, b). Estrogens may play a role in regulating fluid resorption from the efferent ducts during fetal/ neonatal development, and, a role in the gross and functional development of the efferent ducts and rete testis. The specific expression of these aquaporins in the basal or principal cells indicates it might have a role in differentiation, but its role in trans epithelial flow remains elusive.

Water reabsorption in reproductive tract by vas deferens aids in maintaining the luminal environment for sperm maturation. AQP5 localizes to the basal cells of vas deferens (72) (Skowronski et al., 2009). In adult dogs, AQP7 is also expressed in vas deferens and efferent ductules (73)(Domeniconi et al., 2008). Transepithelial water reabsorption mediated via vas deferens maintains the luminal environment suitable for maturation of sperm (74) (Amobi et al.

, 2010). AQP1 not expressed in proximal region of vas deferens in rats, expressed in plasma membrane of epithelial cells in ampulla(31) (Lu et al., 2008).

AQP2 expression was also confirmed in the normal rats, but AQP1&2 differential expression may be due to structural differences along the vas deferens. AQP9 is present throughout the length of the vas deferens (54)

(Pastor-Soler et al., 2001). Aquaporins localize to the plasma membranes of epithelial cells of prostate (AQP1), seminal vesicle (AQP1) and coagulating glands (AQP9); all of which show both secretory and reabsorptive functions(54, 75) (Brown et al.

, 1993; Pastor-Soler et al., 2001). AQP1 localizes to the plasma membrane of epithelial cells of ventral prostate and seminal vesicle(31) (Lu et al., 2008).

In diabetic rats, AQP 1, 3 and 4 in prostrate tissues and seminal vesicles were significantly reduced indicating its role in seminal secretion (76) (Pei et al., 2013). AQP9 and cystic fibrosis transmembrane conductance regulator (CFTR) present in the luminal surface of epididymis aids in the formation of epididymal fluid (65)(Cheung et al., 2003). On treatment with diethylstilbestrol resulted in widening of lumen but there weren’t any changes in AQP9 mRNA expression indicating AQP9 is specifically expressed only in the epithelial cells of efferent ducts and immunohistochemical findings confirmed the same (77)(Wellejus et al., 2008). Upregulation of AQP9 was observed in efferent ducts of male rats on neonatal diethylstilbestrol exposure (77)(Wellejus et al.

, 2008). Castration significantly reduced AQP9 expression in efferent ductules and 3b-diol treatment restored the expression (78) (Picciarelli-Lima et al., 2006). In-vivo and in-vitro, CFTR enhances the water permeability of AQP9 but on inhibition by phloretin and ionidamine the permeability was decreased (65)(Cheung et al., 2003).

Testosterone ameliorates the downregulated expression of AQP9 diethylstilbestrol, GnRHa, ethinyl estradiol, and flutamide (67) (Pastor-Soler

et al., 2010). AQP9 expression in the prostate is controlled by androgens (79) (Wang et al., 2008).

CFTR directly interacts with AQP4 in rat Sertoli cells controlling water transport, any defects/alterations impair spermatogenesis in men (80)(Jesus et al., 2014). Thus, men with cystic fibrosis may have altered aquaporin expression thus leading to infertility/subfertility.