Structure, function, and localization of aquaporins: their possible implications on gamete cryopreservation

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ABSTRACT. The discovery of water channels (aquaporins, AQPs) was a landmark event for the clarification of water transport through the plasma membrane. AQPs belong to a family of intrinsic membrane proteins that act as selective channels for water and for solutes such as glycerol and urea. AQPs were found in different tissues and organs, including male and female reproductive systems. In the swine female reproductive system, the AQPs were localized in the uterus, oviduct, and ovary, as well as in the granulosa cells from primordial follicles. Knowing the involvement of AQPs with the male and female germ cells, as well as their acknowledged role in transporting water through the plasma membrane, the research of these proteins in cryopreservation processes becomes essential. Thus, this review aims to describe the structure and function of AQPs in membranes, highlighting their role

in the reproductive system (male and female). We also discuss the involvement of AQPs in cryopreservation, focusing on the effect and importance of these proteins on the rates of vitrification protocols for preantral follicles present in the ovarian tissue of domestic mammals.

Key words: Aquaporins; Cryopreservation; Intracellular ice formation; Preantral follicles; Cryoprotective agents

INTRODUCTION

The cells of all living organisms are composed mainly of water, a substance with unique features and that is essential for the existence of life on the planet. It is known that water constitutes 95% of blood plasma, which is responsible for carrying oxygen and nutrients to the cells. Water is the principal constituent of human cells and is involved in all physiological and biochemical processes in an organism. Water allows for the regulation of body temperature, is responsible for toxin removal (through urine and sweat), and is the ideal organic solvent through its hydrogen bonding links. In the extracellular environment, water acts as the main biological component, allowing the passage of solutes into different cells in order to maintain turgor pressure (Lehninger et al., 2005). This water exchange between the intra- and extracellular environments occurs through the plasma membrane (Agre and Kozono, 2003) by simple diffusion, despite the polarity difference. However, there are tissues that require fast water movement (kidneys, for example), where the diffusion is simplified by membrane channels (Lehninger et al., 2005).

The plasma membrane consists of a lipid bilayer, carbohydrates, and proteins (Karp, 2002). Membrane proteins have the ability to recognize and allow the transport of substances into the cell such as ions and sugars, and posteriorly expel those from the cell, thus maintaining a lower cytosolic concentration than the surrounding environment (Chandar and Viselli, 2010). This transport can occur by transmembrane channels formed by proteins called aquaporins (AQPs) (Agre and Kozono, 2003). These proteins pass through the lipid bilayer, allowing the passive water movement from side to side of the plasma membrane, which has a hydrophilic channel that is highly specific to the water molecule. Moreover, AQPs have domains facing both the extracellular and cytoplasmic membrane sides (Karp, 2002), as shown in Figure 1.

The family of AQPs has hundreds of members, expressed in bacteria, plants, and animals of different classes, with different sized channels and substance selectivity. This group of proteins transports small solutes with no ionic charge, such as urea and glycerol (Gomes et al., 2009). Over 10 different types of AQPs have been identified in many mammalian systems, including in the reproductive systems of both males and females (Huang et al., 2006). This is an indication that AQPs play an important role in mammalian reproduction (Thoroddsen et al., 2011).

Besides its importance in cellular physiology, it is believed that AQPs play an important role in the process of cryopreservation of gametes (Edashige et al., 2003). This is based on the assumption that AQPs participate in the transport of water through the membrane, a critical point of cryobiology, because an effective cellular dehydration results in the formation of intracellular ice (Mazur, 1970). However, despite its probable influence on cryopreservation, there are few studies demonstrating the action of these proteins during this process.
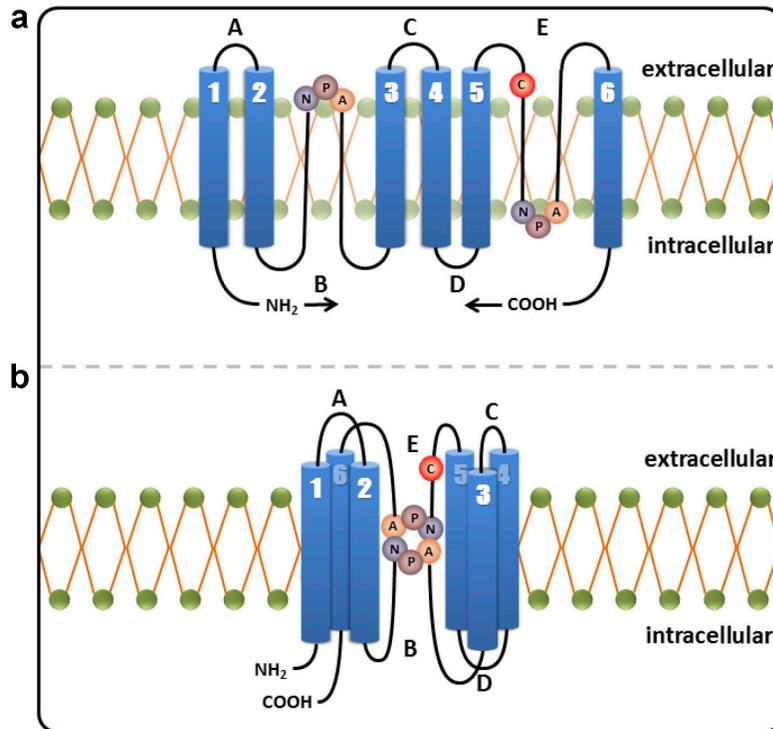
Figure 1. Representation of aquaporin (AQP) structure. **a.** The structure contains five handle connections (A, B, C, D, and E), where E and B contain the conserved motifs NPAs and bend into the molecule forming a narrow portion presenting a hourglass conformation. **b.** Note that in the region of the channel constriction, the NPA motifs are paired. C, in red, represents the cysteine region where the mercury binds conferring sensitivity in most AQPs. (Adapted from Cheidde and Schorr, 1999).

Therefore, based on the hypothesis of AQP involvement in the cryopreservation process, this review aims to describe the structure and function of these proteins in membranes, highlighting their role in the reproductive system (male and female).

PLASMA MEMBRANE AND WATER TRANSPORT

The maintenance of biological organisms is related to its ability to maintain osmotic and ionic balance, adapting to alterations in the environment (homeostasis). The body has regulatory mechanisms that maintain extracellular fluid properties, standard chemical concentrations, and appropriate temperature and pressure conditions, so that the cells are capable of performing physiological functions correctly (Agre and Kozono, 2003). Animal cells have a selective membrane that is responsible for defining and separating the intra- and extracellular environments. This membrane is formed by a lipoprotein bilayer with the role of retaining vital components in the cell, such as oxygen for cellular function, and to regulate the entry and exit of substances such as ions and other solutes (Chandar and Viselli, 2010). With few exceptions, the transport of such molecules occurs through transmembrane proteins. These

proteins are responsible for carrying water molecules, even if these molecules are polar, thereby allowing facilitated diffusion for which a low activation energy ($E_a < 5$ kcal/mol) is used (Fujiyoshi et al., 2002). In addition to transport of molecules, permeability of the membrane to water is also necessary to maintain the osmotic balance. In most cells, water flow occurs freely through the membrane (basal permeability), ensuring the osmotic balance, but in other cells there is a greater control of water flow through the membrane, causing a faster or slower flow rate. The faster flow occurs through proteins called aquaporins (Agre and Kozono, 2003), which are characterized in the next section.

AQUAPORINS

Aquaporins, also known as AQPs, were first reported in 1988 by Denker et al., who purified a large amount of a 28-kDa protein from human erythrocyte membranes. Grounded with these analyses, the authors proposed AQPs to act as pores or channels that allow water and fluids to flow through the membrane (Denker et al., 1988). By the 1990s, homology was detected among these proteins from several species, including microorganisms and plants, but their molecular function was still unknown. Nevertheless, when the expression of these proteins was induced in *Xenopus laevis* oocytes, the coefficient of cell water permeability increased approximately 20 times, verifying the role of these proteins as transmembrane water channels; they were then renamed aquaporin-1 (AQP1), but curiously had a lower coefficient compared with that of other aquaporins. The following year, with the help of complementary DNA (cDNA) technology, the peptide sequences of these transmembrane proteins were determined (Preston et al., 1993); at the time, it was believed that these proteins were associated with the high water permeability of erythrocytes.

Ten years later, AQP1 was found in human nephrons, at the proximal renal tubule and the descending portion of Henle's loop (Agre and Kozono, 2003). Subsequent studies have identified more than 10 different types of AQPs in mammals, found in several organs of different species, such as human kidney (Deen et al., 1994), rat epithelial tissue (Lindsay and Murphy, 2007), human blood vessels (Agre and Kozono, 2003), reproductive system of rodents (McConnell et al., 2002), dogs (Domeniconi et al., 2007), and pigs (Skowronski et al., 2009). The studies with AQP1 demonstrated that these proteins are small and integral membrane protein types, crossing the membrane from side to side, and containing two semi-pores that together form a water channel (Verkman and Mitra, 2000), assisting the transport of solutes (Thoroddsen et al., 2011).

In addition to the transport of these substances, most AQPs in mammals contain extracellular sites susceptible to mercury chloride ($HgCl_2$), as well as other metals such as silver nitrate ($AgNO_3$) and gold (Au). Such components bind to a cysteine residue, more specifically sulfide groups, inhibiting it, causing a conformational change, and subsequently reducing the water flow (Wan et al., 2004). However, this property is not a general feature for all AQPs; AQP4 and AQP7, for example, have an alanine bound to the cysteine at the position of the $HgCl_2$ binding site, precluding their sensitivity (Cheidde and Schor, 1999).

Structural characterization of aquaporins

Structurally, AQPs are a class of porins that forms channels, with molecular masses between 28 and 30 kDa (Denker et al., 1988). These proteins have six domains, connected by

five loops, as shown in Figure 1. In this structure, each polypeptide is formed by a single chain with about 270 amino acids, has terminal amino and carboxyl groups, and is always located in the cell cytoplasm (Preston et al., 1993). AQPs also have three extracellular loops (A, C, and E) and two intracellular loops (B and D) (Fujiyoshi et al., 2002) with the asparagine-proline-alanine sequences fairly conserved, forming short helices and are hydrophobic from opposite sides, associating with substrates selectivity. As shown in Figure 1, most members of this protein family possess a cysteine residue (Cys 189) in the E loop (extracellular), which is situated near the channel responsible for functional sensitivity to mercury, which blocks its function (Preston et al., 1993).

Hydrophilic asparagines are at the center of the channel, each pairing with its homologous group from the opposite loop and forming the pore region (narrowest point of the channel) of about 3 Å (Agre and Kozono, 2003). This pore has electrostatic interactions, where the water molecule, while in Brownian motion (random movement) in the extracellular environment, renders the AQP outer cone walls in a hydrophobic state, causing repulsion (Romeiro et al., 2006). Present in the narrowest region of the cone are two terminal unsaturated amines, which favor hydrogen bonds binding. . Thus, the water dipole binding is aligned with two asparagine molecules and then the molecule crosses the channel (Jensen et al., 2003). This transport is arranged in an iso-osmolar order, at no ATP expense (Jensen et al., 2003).

Some AQPs are also known as aquaglyceroporins (GLPs); the basic difference between these proteins and AQPs is the pore size, a region also known as a selective filter. In some AQPs, the pore measures ~2.8 Å, whereas in GLPs, it reaches ~3.4 Å. In general, AQPs present a selective region to water (polar region) and contain conserved histidine residues, whereas GLP regions are hydrophobic with two aromatic residues conserved, usually tryptophan and tyrosine. In this sense, the AQP architecture is well preserved, and the specific side-chain pores modulate functional differences (Savage and Stroud, 2007).

Main types and functions of aquaporins

Based on their permeability, mammalian AQPs are divided into three groups: a) water-selective permeable aquaporins (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8); b) GLPs permeable to water, glycerol, urea, and other solutes (AQP3, AQP7, AQP9, and AQP10); c) subcellular aquaporins or super-aquaporins (AQP11 and AQP12), which have low homology with the other AQPs (Murai-Hatano et al., 2008). Aquaporin-0 (AQP0), previously known as the main intrinsic protein, can be found in the lenses of several species; for instance, rodents, cattle, and humans. The lens accommodation in the eye is accompanied by significant volume alterations, which requires the rapid flow of water, with the cell membrane pores ensuring the needed velocity for hydrotransport. Furthermore, AQP0 also seems to act on the local eye microcirculation, since the lens is devoid of blood vessels. The evidence, apart from the involvement of AQP0s in water transport, suggests that this protein also plays a structural role, acting in cell adhesion; that is, gaps in the joints of the fiber lens (Harries et al., 2004). Unlike other aquaporins, the AQP0 permeability to water is remarkably low, around 40 times lower than the inside AQP1 lens (Liu et al., 2011).

AQP1 was the first of these proteins to be characterized in human erythrocytes (Preston et al., 1993). It is expressed mainly in the red blood cells, brain, lungs, and kidneys and is associated with water reabsorption and fluid secretion. Among the identified kidney aquaporins (AQPs 1, 2, 3, 4, and 6), AQP1 is expressed the most, particularly in the proximal tubule

in the ascending branch, which delivers the route leading to the resorption of water in the proximal nephron (Nielsen et al., 1999). This protein is also expressed in the capillary endothelium, reaffirming its importance for water transport in humans.

AQP2 is expressed mainly in the kidney, and its protein mutation is related with diabetes insipidus (Deen et al., 1994). These mutations are located in the chromosome region 12q13, leading to a deviation or disruption of the function of water channels, affecting both men and women. Studies are thus proving that AQP2 is the only water channel whose function is regulated (Cheidde and Schor, 1999). This same protein was expressed in the endolymphatic sac in the ear in humans, suggesting an active role in the homeostasis of the endolymph (Ishibashi, 2009). AQP2 was also found in the rete testis, efferent ducts, and epididymis of dogs, although its functional role in the reproductive tract is still unknown (Domeniconi et al., 2007).

AQP3 has been found in a wide variety of organs such as the kidneys (Ishibashi et al., 1994), urinary system, digestive system, respiratory system (King et al., 2001), and skin and eyes (Hamann et al., 1998). In most cases, this protein is expressed in epithelial cells or on the lumen surface. Using *X. laevis* oocytes, several studies showed that this protein carries glycerol and other substances such as ethylene glycol, propylene glycol and acetamide (Yamaji et al., 2006), reasons why it is considered a GLP. Beyond these observations, the permeability and hydraulic conductivity of the channel were evaluated, where it was found that AQP3 plays an important role in transporting ethylene glycol, propylene glycol, and glycerol during bovine embryo cryopreservation.

Regarding AQP4, it is known that this protein is involved with water balance regulation in the brain, since in cases of cerebral edema (ischemic, hypo-osmolar stress-related, tumor-related, traumatic, and inflammatory), there is an increased expression of this protein (King et al., 2001). In humans, an improved expression of AQP4 was confirmed in cases of cerebral ischemia, glial tumors, traumatic brain injury, infection, and inflammatory diseases of the central nervous system (Saadoun et al., 2002). Other authors have proposed that a greater understanding of the molecular channel opens a new dimension for action in the field of brain edema and makes possible the emergence of new therapeutic methods.

As for AQP5, it is mainly expressed in the alveolar epithelium, trachea, and upper bronchus (Gomes et al., 2009). AQP5 has a major task in maintaining water homeostasis in the lungs (Verkman, 2005) and can also regulate the release of saliva in the oral mucosa (Tsubota et al., 2001), and tears and salivary glands (Steinfeld et al., 2001). This protein was also detected in corneal cells, maintaining the epithelium transparency, as well as on its underlying stroma. Interestingly, the expression of AQP5 in the rat uterus increased more rapidly after the administration of estrogen (Ishibashi, 2009).

Regarding AQP6, studies revealed that this protein is present in the membranes of intracellular vesicles, transporting mainly nitrate, and in the renal duct, it demonstrated permeability to some ions such as the chloride ion (Gomes et al., 2009). The special feature of AQP6, proven through transmission electron microscopy, is that this protein is located not on the cell surface but in intracellular vesicles of the renal collecting duct epithelial cells.

AQP7 molecules are involved in water permeability, as well as solutes. This protein is expressed mainly in the adipose tissue, testis, heart, skeletal muscle, and kidneys. In adipose tissue, the presence of AQP7 seems to be tied to a greater ease of glycerol secretion (Rodriguez et al., 2006). AQP7 also transports nonionic solute surfactants with low molecular weight. In follicular maturation, studies have confirmed the presence of this protein in granulosa cells, and it is believed to be related with antrum formation (Huang et al., 2006).

AQP8 is expressed in many organs and tissues, especially the pancreas, liver, salivary glands, kidneys, testes, epididymis, duodenum, stomach, jejunum, lung, trachea, and placenta (Liu et al., 2006). The induction of this recombinant protein in rats, mice, and humans demonstrated its action on carrying water; additionally, in rats and humans, AQP8s are not permeable to urea and glycerol. Furthermore, in humans, AQP8 is permeable to ammonia analogs (formamide and methylammonium), suggesting that this AQP can carry *in vivo* ammonia, contributing physiologically to the acid-base balance (Liu et al., 2006).

AQP9 has been identified in the liver, leukocytes, testis, brain, and ovary (Gomes et al., 2009) and is involved in the permeability of water and small solutes. Its role in the brain is still unknown; however, its permeability to lactate and glycerol indicates that this protein may be important in energy metabolism (Tait et al., 2008). AQP9 was also localized in granulosa cells from developing follicles in the oviduct and uterus of sows (Skowronski et al., 2009).

AQP10 was found in the small intestine of humans, particularly in the jejunum (Ishibashi et al., 2002), presenting the same function as GLPs, and is also present in absorptive epithelial cells. Interestingly, a study in mice revealed that AQP10 is a pseudogene. In an attempt to elucidate the physiological significance of this protein, the authors have cloned the AQP10 gene and the results showed that this gene was not detected in the jejunum or, indeed, any other tissue of that species. Additionally, it was revealed that the gene in charge of AQP10 expression in mice contains several mutations, which leads to production of a non-functional protein. Another feature of this protein, in humans, is that their absorption is inhibited in the presence of glycerol derivatives, such as ethylene glycol and propylenediol (Ishii et al., 2011). However, this mechanism is not yet fully elucidated, requiring further research.

AQP11 and AQP12 belong to a new aquaporin subfamily called super-aquaporins (Ishibashi, 2009). AQP11 is expressed in the kidneys, brain, liver, testes, heart, intestine, and adipose tissue (Gorelick et al., 2006), but its function is still unknown (Buffoli, 2010). Interestingly, when AQP11 was expressed in *X. laevis* oocytes, there was no water transportation (Gorelick et al., 2006). On the other hand, when the protein was reconstituted into liposomes, a channel of high activity to water was evident. Currently, the reason for this discrepancy is not yet clear, requiring further study to understand AQP11's function within the cell. AQP12 is found only in pancreatic acinar cells (Buffoli, 2010). The specific location has not been established for this subfamily, but its presence in the pancreas implies a possible pathway in digestive enzyme secretion and pancreatic cell fluids (Rojek et al., 2008). AQP12 has a higher identity with AQP11 (32%) compared with other AQPs, which show less than 15% identity (Ishibashi, 2009). A structural feature of AQP12 is the lack of an N-terminal cytoplasmic region, in contrast to all other known aquaporins (Ishibashi, 2009). Table 1 shows a summary of the location of all AQPs found in various mammalian species, which were based on polymerase chain reaction, Northern blot, or microarray results, and have not necessarily been confirmed by immunohistochemistry.

AQUAPORINS AND THE REPRODUCTIVE SYSTEM

As described above, the AQPs can be found in many mammalian systems. In particular, AQPs have been found in various organs of the reproductive system, regardless of sex (Jablonski et al., 2003), thereby demonstrating its function in mammalian reproduction.

Table 1. Distributions of aquaporins (AQPs) in different tissues in mammals.

Gene	Tissue/cellular localization	Species	Reference
AQP0	Crystalline (eye)	Rodent, bovine and human	Liu et al., 2011
AQP1	Erythrocytes, brain, lungs, kidneys, proximal nephron, capillary endothelium, ovary, semen, ovary, oviduct, and uterus	Human, rodent, swine, canine	Preston et al., 1993; McConnell et al., 2002; Domeniconi, 2007; Skowronski et al., 2009
AQP2	Duct renal, ovarian, testicular network, efferent ducts, and epididymis	Human, rodent (rats), canine	Deen et al., 1994; Domeniconi et al., 2007
AQP3	Kidneys, urinary tract, digestive tract, respiratory tract, skin, eyes, reproductive system, germ cell, and ovarian	Human, rodent (rat and mouse)	Yamaji et al., 2006
AQP4	Brain, central nervous system, and ovary	Human	Romeiro et al., 2006
AQP5	Alveolar epithelium, tracheal and bronchial superior cornea, ovary, uterus, and oviduct	Swine, rodent (rat)	Gomes et al., 2009
AQP6	Membranes of intracellular vesicles and renal duct	Rodent (rat), human	Gomes et al., 2009
AQP7	Adipose tissue, testis, heart, skeletal muscle, kidney, and follicle (granulosa cells)	Rodent (rat)	McConnell et al., 2002; Huang et al., 2006
AQP8	Pancreas, liver, salivary gland, kidney, testis, epididymis, duodenum, stomach, jejunum, lung, trachea, and placenta	Rodent (rat and mouse)	Liu et al., 2006
AQP9	Liver, leukocytes, testis, brain, ovary, oviduct, uterus, and sperm	Rodent (rat and mouse, swine, canine)	Tait et al., 2008; Skowronski et al., 2009
AQP10	Human small intestine (specifically in the jejunum)	Human, mouse (pseudogene)	Ishibashi et al., 2002; Ishii et al., 2011
AQP11	Kidney, brain, liver, testis, heart, intestine, adipose tissue, and testis	Rat, human	Ishibashi, 2009; Yakata et al., 2011
AQP12	Pancreatic acinar cells	Mouse, monkey	Ishibashi, 2009

Male reproductive system

AQPs are found in the intra- and extratesticular spermatic pathways (Domeniconi et al., 2007). In the male reproductive system, several AQPs have been expressed in different locations, such as the testes (AQP0, AQP1, AQP7, AQP8, and AQP9), efferent ducts (AQP1, AQP9, and AQP10), epididymis (AQP1, AQP3, AQP9, and AQP10), vas deferens (AQP1, AQP2, and AQP9), and accessory glands (AQP1 and AQP9) of adult mammals (Huang et al., 2006). In addition to these, AQPs 3 and 10 have also been described in rats (Hermo et al., 2004). Recent studies detected two new AQPs in mammals, AQPs 11 and 12, with AQP11 being present in the testes and kidney, whereas AQP12 is selectively expressed in the pancreas (Ishibashi, 2009).

Among the GLPs, AQP3 is located in the epididymal basal cells (Hermo et al., 2004). AQP7 is located throughout the tail of the sperm, except at the end, as reported in humans. The expression of this protein was also found in germ cells (spermatids) of adults and developing rats (Huang et al., 2006), and in testicular and epididymal spermatozoa (Calamita et al., 2001), indicating a possible role in storage of sperm maturation as well as in late spermatogenesis. However, in non-sterile knockout mice, semen analysis demonstrated no morphological or functional abnormalities, suggesting that this AQP is turned on and off at different times (Sohara et al., 2009).

In rats and mice, AQP9 was expressed in immature germ cells (Tsukaguchi et al., 1998), efferent ducts, and over the epididymis and vas deferens of dogs, showing that this protein is abundantly expressed in the testis (Domeniconi et al., 2007). In the epididymal cells, AQP9 was expressed in greater quantities in the initial and caudal segments of the epididymis (Badran and Hermo, 2002). These AQPs as well as AQP1, present in the efferent duct and epididymis, may be involved in fluid absorption, promoting sperm concentration, suggesting for this epithelium a great potential related to the movement of water and small solutes along the epididymis (Hermo and Smith, 2011).

On the other hand, AQP10 was found in the epithelium of efferent ducts (Hermo et al., 2004). In the literature, there are no studies associating AQPs with fertility or infertility in men. However, these studies may be the key to uncovering the interaction of these proteins to the maintenance of the germinal epithelium, the powerful functionality of sperm production, and natural fertilization.

Female reproductive system

The first reports of the presence of AQPs in the female reproductive system occurred in the human uterus, after cDNA isolation and sequencing. These authors presented that the cloned cDNA had 99.8% homology to the AQP1 found in human erythrocytes. Among the AQPs found, nine isoforms have been expressed in the female reproductive system of humans, rats, and mice (Huang et al., 2006). Studies have demonstrated the expression of AQPs in the uterus of rats (AQPs 5 and 9, Lindsay and Murphy, 2007), mice (AQPs 0, 1, 4, and 5, Richard et al., 2003; AQPs 1, 3, and 8, Jablonski et al., 2003), pigs (AQPs 1, 5, and 9, Skowronski et al., 2009), and humans (AQPs 1-4, Thoroddsen et al., 2011). AQP1 expression was detected in the rat uterus, suggesting that these proteins represent a control system for water movement across the intraluminal, interstitial, and capillary compartments (Skowronski et al., 2009).

In the oviduct of female rats, the presence of AQPs 5, 8, and 9 was observed only in the epithelial cells. In addition, studies found that AQP9 was the only AQP localized after ovariectomy and administration of progesterone and estradiol (Branes et al., 2005). These results indicate that AQPs function not only in water transport but also in the absorption or secretion of luminal fluid metabolites, thus maintaining a suitable environment for reproductive functions (Kenny et al., 2002). During the estrous cycle of rats, a variation of AQP9 was observed, with higher levels developing during the proestrus and estrus phases. This finding indicates that steroid hormones can regulate the expression of this protein and thus control water transport into the oviduct lumen (Branes et al., 2005).

In a study conducted by Skowronski et al. (2009), only three of the AQPs (1, 5, and 9) were expressed in the female reproductive organs. AQP1 was localized in the ovary, oviduct, and uterus. AQP5, besides being present in these organs, was also localized in granulosa cells of primordial follicles, smooth muscle and luminal cells, and glandular epithelial cells. AQP9 was already observed in the granulosa cells of developing follicles, in the oviduct and uterus, and in ovarian epithelial cells. This study also suggested that the ovarian AQP9 does not control water transport only. The strong expression of this protein in ovarian tissues could have an important implication in follicular development, because it is a GLP and therefore allows the passage of other electrolytes. In this case, the protein could ensure a sufficient supply of estrogen to granulosa cells, making it essential for follicle development (Skowronski et al., 2009).

According to the literature, it is assumed that sex hormones may directly regulate the transcription of AQP genes, but the effect may be mediated through an indirect route that involves the expression of other intracellular mediators. The permeability regulators that vary along the reproductive cycle, such as the transcription from receptors to the vascular endothelial growth factor, and the receptor for the platelet activation factor expressed by epithelial cells of the uterine tube, are possible explanations for this mechanism (Jablonski et al., 2003). There is evidence that AQPs may participate in the regulation of steroid hormones produced by ovarian follicles (Jablonski et al., 2003; Huang et al., 2006). However, the limited results about AQPs in the ovary suggest that its main function in this organ is in antrum formation and expansion (Thoroddsen et al., 2011).

The exact mechanism for the fluid transport from layer to layer of teak granulosa cells and the formation of avascular follicular fluid is not yet completely understood (Rodgers and Irving-Rodgers, 2010), but it is a slow process and not likely to occur by simple diffusion. Studies indicate that the AQPs present in these follicles were responsible for the rapid passage of a large volume of water into the antral cavity (Preston et al., 1993).

A study of the human ovary verified AQPs (1-4) expressions at different times of the preovulatory follicle, in the theca and granulosa cells, and observed their expressions in the follicles during ovulation. In this same study, there was a sharp increase in AQPs 2 and 3 at the beginning of ovulation, suggesting a role in follicular rupture, and a high expression of AQP1 at the end of this event, indicating its function in corpus luteum formation. Thus, it is believed that the AQPs also exert a critical key role in ovulation (Thoroddsen et al., 2011). In Figure 2, we can see the presence of this protein at different times of folliculogenesis.

IMPORTANCE OF AQUAPORINS TO CRYOPRESERVATION OF MALE AND FEMALE GAMETES

In the last decade, the advances in understanding about the cryobiology phenomenon,

which aims at preserving biological material for an undetermined time in low temperatures (Mazur, 1970), contributed to the development of accurate methods for the preservation of cells, mainly related to gametes. The cryopreservation of mammalian gametes and embryos has been used successfully in many aspects, including the preservation of the genetic variability in laboratory animals, the reproduction of production animals, and assisted reproduction in human beings (Rall, 2001).
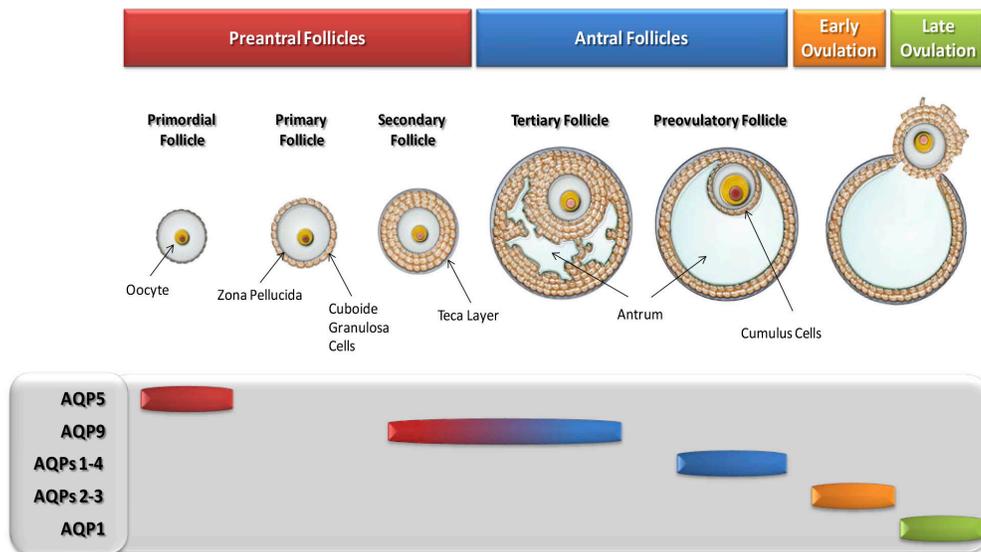

Figure 2. Attendance of aquaporins (AQPs) in folliculogenesis. AQP5: in granulosa cells in porcine (Skowronski et al., 2009), AQP9: in granulosa cells (Skowronski et al., 2009), AQPs 1-4: ovulation period, AQP1: final ovulation (Thoroddsen et al., 2011).

The process of cryopreservation can be achieved by using two methods: slow freezing and vitrification. With regard to slow freezing, this is based on cell dehydration, the association of a soft osmotic balance, the use of solutions that contain low concentrations of cryoprotectant agents (CPAs), and a gradual reduction of temperature, usually by using a programmable freezer. Vitrification involves a fast substitution of the intracellular water with solutions containing high concentrations of CPA, and an abrupt reduction of temperature, aiming for the formation of a vitreous state without the formation of ice crystals. Regardless of the method of cryopreservation used, the addition of CPAs is indispensable to providing cell cryoprotection, allowing the cells to be able to hold up to the injuries inherent in the procedures of cryopreservation (Santos, 2005). However, cell cryopreservation in large amounts and of multicellular systems still continues to be a challenge in some cases, owing to intracellular ice formation (IIF), the toxicity of the CPAs, and the risks of osmotic shock, especially at the moment of thawing and removal of the cryoprotectant agent (Mazur, 1984).

Specifically, in relation to IIF, it is hypothesized that these crystals may be able to form and grow themselves through the canals (AQPs) formed in the membrane, although there is evidence that the ice formed has a larger size in comparison with these canals (Mazur,

1970). In the process of cryopreservation, there are two moments that are crucial to the IIF. One of these is when the CPA is added (balance period), especially in the slow freezing process, wherein there has to be a way for the water to exit (cellular dehydration) concurrently with the CPA entrance in order to avoid the formation of new crystals or even the increase of small crystals inside of the cell or the tissue. Another moment is the thawing, which must take place quickly in order to avoid the formation of new crystals or even the growth of small crystals previously formed (Mazur, 1970). Thus, taking into consideration the IIF hypothesis, and based on the need for maximum removal of the intracellular water before the process of cryopreservation, especially in the process of slow freezing, associated with the fact that the AQPs accelerate the transportation of water in the cell, it is acknowledged that these proteins perform a role in the transportation of water, which is extremely important in the process of cellular cryopreservation.

As described in the sections above, some AQPs are permeable to glycerol and urea, as well as to metalloids and a large variety of non-charged solutes (carbamide, polyols, purines, pyrimidine), including the CPAs, such as propyleneglycol, ethyleneglycol, acetamide, and, possibly, dimethyl sulfoxide (Chauvigné et al., 2011). This factor is extremely important, considering that during the balance period or during CPA removal, there must be a perfect control of the water and CPA flow through the cellular membrane; that is, the water leaving and the CPA entering in the first case, and the water entering and CPA leaving in the second case.

A study using mice demonstrated the expression of AQP3 in mature oocytes (metaphase II) and in embryos at various stages of development. With regard to the cryopreservation of these structures, the authors reported that this protein performs a significant role in the transportation of water, as demonstrated by the use of artificial AQP3, which increased the permeability to water as well as to glycerol. The result of this effect was the increase of oocyte surviving after vitrification. In addition, in this study, the hydraulic conductivity and the glycerol permeability were also evaluated, confirming that the formulation of the artificial AQP3 improved the permeability of the cryoprotectant agent (Edashige et al., 2003).

The studies involving these proteins in the cryopreservation of mammals are still scarce and in the initial stages. Thus, it is extremely important to conduct new studies to assess the effects of AQPs in this process, with the aim to perfect this technique and consequently to increase the success rates of cryopreservation of embryos and gametes.

FINAL CONSIDERATIONS

As discussed, the AQPs are proteins existing in different mammalian tissues and that have the main function of water transportation, although they can perform other specific functions. In the reproduction system, for example, they can be in various locations independently of gender. In males, AQPs are related to the motility, maturation, and storage capacity of sperm. In the case of the female system, AQPs play important roles in follicle development and the maintenance of female fertility. Knowing the involvement of AQPs in the female and male germ cells, as well as their recognized role in water transportation across the plasma membrane, it is essential to investigate this family of proteins in the process of cryopreservation of these structures.

Despite the fact that gene analysis and expression are essential for the understanding of tissue physiology, there are no mammalian studies being conducted to examine the gene

expression of proteins involved in the important processes for survival after cryopreservation. In the survival process, the exposure of cells to the extreme cold can result in irreparable injury caused by the formation of intracellular ice crystals, which must be avoided or minimized. It is hypothesized that during the formation of intracellular ice, external ice makes contact with the ice surface on the cells and grows through preexisting pores in the membrane of the cell to the interior of the nucleated cell, a process caused by the presence of the AQPs (Mazur, 1984). In attempts to solve this problem, CPAs are used but they also increase the risk of cellular toxicity. However, regardless of how crystals of ice are formed in the interior of cells, the formation of these crystals must be avoided through removal of the maximum possible quantity of intracellular water (Faustino et al., 2011). Therefore, whether or not aquaporin expression is involved in the efficiency of this process must be assessed, and consequently a positive effect will be made in relation to the viability of gametes after cryopreservation, contributing in a significant way to the perfection of cryopreservation protocols.

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