Opinion article

Aquaporins in male reproductive system: a chance for paternity or a road to nowhere?

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Abstract

Aquaporins (AQPs) – small, "unusual" proteins, whose discovery revolutionized the view of membrane transport of water and other small molecules, are essential for all living organism. AQPs located in the male reproductive system seem to play a key role in the proper course of many processes occurring within it, and thus maintaining a high reproductive potential.

There is no need to convince anyone that the global decline in male reproductive potential observed in recent years has become one of the most serious obstacles on the way to the longed-for fatherhood. Often, despite the lack of abnormalities in both the structure and functioning of the male reproductive organs and with the correct sperm parameters, fertilization does not take place, even with the use of modern procedures. Hence, many authors and medical practitioners have long emphasized that the current standards and recommendations for diagnostic testing of male reproductive tract and semen quality parameters are not sufficient and new markers should be sought to enable precise determination of male reproductive potential.[1] Could aquaporins (AQPs) – small, transmembrane proteins permeable to water and other small molecules, which are widely distributed throughout the body, be a breakthrough in research on the causes of male fertility disorders? Could the measurement of these proteins become a modern and effective tool in the diagnostics of male infertility in the future? These

questions seem to currently trouble many scientists working in the broad field of reproductive method improvements both in humans and animals.

Of the 13 aquaporins known in mammals (AQP0-AQP12), the presence of as many as 11 have been confirmed in the male reproductive system (Figure 1A). It should be noted that these are two more aquaporins compared to the kidneys, which, as is generally known, regulate the water and electrolyte balance of the whole organism. The mere presence of 11 AQPs in male sex organs indicates the potentially important role of these proteins in the normal course of reproductive processes. According to many authors, AQPs are involved in spermatogenesis, luminal fluid reabsorption/secretion and sperm physiology, including cell volume control, osmoadaptation and motility.[2] It is also worth mentioning that AQPs expression in the male reproductive tissues may be under the control of sex steroid hormones, for example, AQP9 levels in the developing rat epididymis was downregulated by estrogens, but this effect could be reversed by testosterone administration.[3] Currently, aquaporins located in sperm attract the most attention. To date, AQP3, AQP7, AQP8 and AQP11, which are present at specific sites in the sperm, have been observed in humans and many animal species (Figure 1B).[4] A broader analysis has not been carried out so far, and it has not been verified whether the remaining from 13 aquaporins may also be expressed in spermatozoa. After ejaculation, the sperm have to travel a long way before reaching their destination in the female's reproductive tract in a hypoosmotic medium. The hypoosmotic environment triggers a series of changes in sperm, in which AQPs appear to play essential roles. According to Delgado-Bermudez et al. (2022) after entering the female tract, sperm cells swell, as a result of rapid water influx, and their volume increases, resulting in destabilization of the cell membrane.[5] The proper sperm volume is restored relatively quickly, but it is the destabilization of the membranes caused by excessive water uptake that triggers the entrance of calcium and bicarbonate, which activates sperm capacitation through the protein kinase A (PKA) signaling pathway that drives hyperactive motility and prepares the sperm to the acrosome reaction. Undoubtedly, these changes would not be possible without aquaporins, which, located mainly in the sperm cell membrane, enable efficient water transport in both directions. In addition to water, AQPs transport glycerol (AQP3, AQP7 and AQP11) necessary for energy production and H_2O_2 (AQP3, AQP8 and AQP11), whose excess effectively impairs the fertilization-enabling capacitation processes. With these facts in mind, it seems easy to predict the effects of decreased expression of AQPs in sperm. More than a decade ago, it was shown that while lower expression of AQP3 did not affect the motility, it caused increased vulnerability to hypotonic cell swelling, characterized by tail deformation.[6] It was also found that lower expression or lack of AQP7 was related to abnormal sperm

morphology and reduced motility.[7] In turn, the deficiency of AQP8 in mitochondrial membranes hinders the elimination of reactive oxygen species (ROS).[8] Although there are many other data available in the literature that indicate the importance of AQPs in proper sperm function, no attempts have been made to date to create reference standards for both their localization and expression. Currently, we have methods, such as immunofluorescence or Western blot that can evaluate the distribution of these proteins in sperm relatively easily, inexpensively and rapidly. Although the development of reference standards for healthy men seems to be very promising and offers a wide range of opportunities, some doubt the sense of conducting research in this area and trying to introduce the determination of AQPs in sperm into everyday medical practice. This is mainly due to reports from studies conducted in knockout animals. It is indeed puzzling that no significant abnormalities are observed in genetically modified laboratory animals lacking AQP1, AQP3, AQP4, AQP7, AQP8 and AQP9, and these animals are still fertile. It should be emphasized that there is no information regarding fertility in mice associated with depletion of AQP11, as the animals suffer from polycystic kidney disease and die in the first weeks of life. How is it possible that the lack of AQPs does not significantly affect male reproductive processes in transgenic animals? According to some authors, this is related to the ability to produce a series of changes that compensate for and increase membrane water transport and other small solutes. However, one cannot entirely agree with this notion, because, first of all, there is no detailed information on the analysis of sperm parameters and other important fertility indicators in these animals that could unequivocally confirm this. Second, the currently available data indicate a reduction in fertility and effective fertilization, especially in mice with AQP3 depletion.[6]

Another very interesting aspect related to the determination of AQPs in sperm is their potential importance in semen cryopreservation. During freezing-thawing processes, sperm must adapt to rapid osmotic changes and precisely adjust the flow of water and glycerol to limit damage to the membrane and organelles.[1] It is not a coincidence that out of 4 aquaporins identified in sperm, 3 of them are permeable to glycerol. In addition to being a source of energy and an important element in sperm metabolism, glycerol is known for its cryoprotective properties. In both humans and animals, there are individual differences in cryoresistance and the ability of sperm to survive in cold temperatures. Does post-freezing semen quality depend on AQPs expression in sperm? Could measuring the expression of these proteins be useful in the future to predict tolerance to freeze-thawing in spermatozoa? Semen cryopreservation is of great importance in large-scale livestock farming, hence the currently conducted research in this area mainly concerns these species. A trend is emerging from the many works published

so far, as most authors have noted a positive relationship between the expression of AQPs in sperm and the quality of cryopreserved semen. Among them, Fujii and coworkers (2018) demonstrated that the relative abundance of AQP3 and AQP7 was positively associated with motility and velocity after sperm freeze-thawing.[9] The results obtained so far in animals appear to be very promising and prompt this type of research also in humans. Introducing the determination of AQPs levels in sperm into everyday practice will not only be helpful in fully determining the quality of semen, but also in assessing its suitability for cryopreservation. It should be noted that the pharmacological modulators of selected AQPs have long been sought for and the first information on them has become available, which creates further opportunities not only related to diagnostics, but also appropriate future therapy.

In conclusion, it should be definitely stated that the modern man struggling with the problem of infertility needs state-of-the-art and extended diagnostics, as well as therapy "tailored" to current times. Are AQPs the answer to these challenges or a dead end? Regardless of the arguments for and against, as long as the answer to this question is not experimentally verified, the discussion on this topic can go on indefinitely.

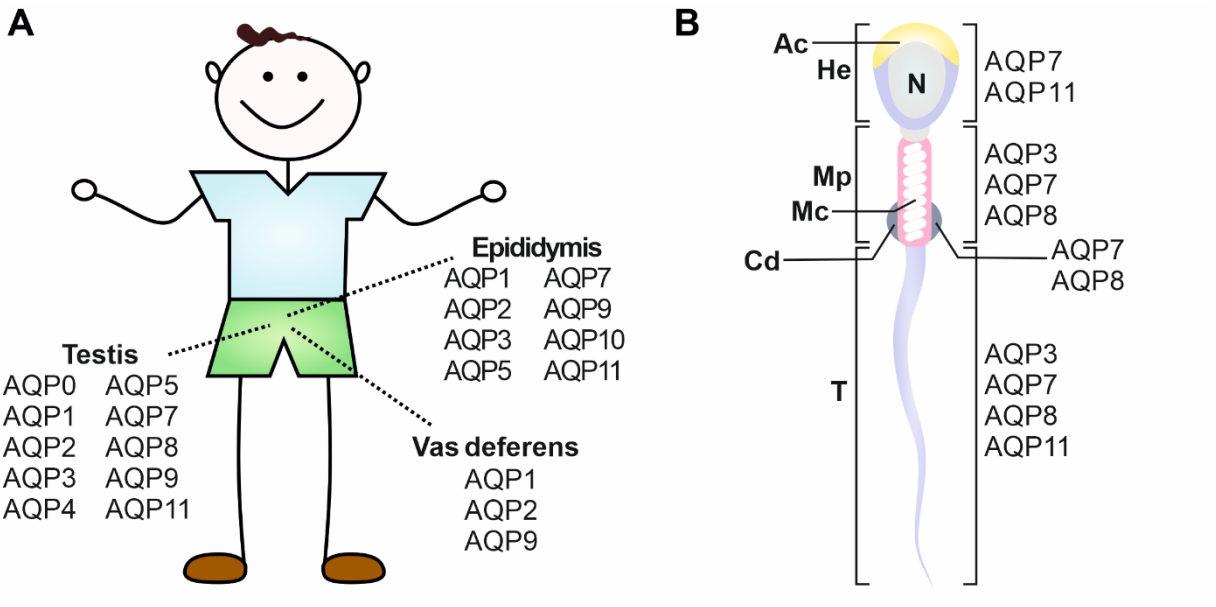

Figure 1. Distribution of aquaporins (AQPs) in human and animal (**A**) reproductive tracts (modified from Zelenina et al. 2005)[10] and (**B**) sperm (modified from Michałek et al. 2021, Oberska and Michałek 2021)[1,4]. Both diagrams show the generalized and most frequently observed locations of individual AQPs. Abbrevations: Ac, acrosome; He, head; Mp, mid-piece; Mc, mitochondria; Cd, cytoplasmic droplet; T, tail.

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Author contributions

KM: Conceptualization; Writing-original draft (lead); Project administration; Funding acquisition. PO: Writing-original draft; Visualization.

Conflict of interest

The authors have no conflict of interest to disclose.

References

1. Michalek K, Oberska P, Malkowska P, Bartkiene E. In search of new potential markers for male fertility and semen quality control. Aquaporins in reproductive system and metabolomic profiling of semen. J Physiol Pharmacol 2021;72:300-319. https://doi.org/10.26402/jpp.2021.3.01
2. Ribeiro JC, Alves MG, Yeste M, Cho YS, Calamita G, Oliveira PF. Aquaporins and (in)fertility: More than just water transport. Biochim Biophys Acta Mol Basis Dis 2020;1867:166039. https://doi.org/10.1016/j.bbadis.2020.166039
3. Pastor-Soler NM, Fisher JS, Sharpe R, Hill E, Van Hoek A, Brown D, Breton S. Aquaporin 9 expression in the developing rat epididymis is modulated by steroid hormones. Reproduction 2010;139:613-621. https://doi.org/10.1530/REP-09-0284
4. Oberska P, Michałek K. Aquaporins: New markers for male (in)fertility in livestock and poultry? Anim Rep. Sci. 2021;231:106807. https://doi.org/10.1016/j.anireprosci.2021.106807
5. Delgado-Bermúdez A, Ribas-Maynou J, Yeste M. Relevance of Aquaporins for Gamete Function and Cryopreservation. Animals (Basel) 2022;12:573 https://doi.org/10.3390/ani12050573
6. Chen Q, Peng H, Lei L, Zhang Y, Kuang H, Cao Y, Shi QX, Ma T, Duan E. Aquaporin3 is a sperm water channel essential for postcopulatory sperm osmoadaptation and migration. Cell Res. 2011;21:922-933. https://doi.org/10.1038/cr.2010.169

7. Saito K, Kageyama Y, Okada Y, Kawakami S, Kihara K, Ishibashi K, Sasaki S. Localization of aquaporin-7 in human testis and ejaculated sperm: possible involvement in maintenance of sperm quality. J Urol 2004;172:2073-2076. https://doi.org/10.1097/01.ju.0000141499.08650.ab
8. Laforenza U, Pellavio G, Marchetti AL, Omes C, Todaro F, Gastaldi G. Aquaporin-Mediated Water and Hydrogen Peroxide Transport Is Involved in Normal Human Spermatozoa Functioning. Int J Mol Sci 2017;18:66. https://doi.org/10.3390/ijms18010066
9. Fujii T, Hirayama H, Fukuda S, Kageyama S, Naito A, Yoshino H, Moriyasu S, Yamazaki T, Sakamoto K, Hayakawa H, Takahashi K, Takahashi Y, Sawai K. Expression and localization of aquaporins 3 and 7 in bull spermatozoa and their relevance to sperm motility after cryopreservation. J Reprod Dev 2018;64:327-335. https://doi.org/10.1262/jrd.2017-166
10. Zelenina M, Zelenin S, Aperia A. Water channels (aquaporins) and their role for postnatal adaptation. Pediatr Res 2005;57:47-53. https://doi.org/10.1203/01.pdr.0000159572.79074.0b