IDENTIFYING THE FUNCTIONAL ROLE OF AQUAPORINS IN MAMMALIAN SPERM CRYOPRESERVATION

Ariadna Delgado-Bermúdez [1], Marc Llavanera [1], Jaime Catalán [2], Sergi Bonet [1], Jordi Miró [2], Marc Yeste [1]

[1] *Biotechnology of Animal and Human Reproduction (TechnoSperm), Department of Biology, Faculty of Sciences and Institute of Food and Agricultural Technology, University of Girona, Girona, Spain*

[2] *Equine Reproduction Service, Department of Animal Medicine and Surgery, Faculty of Veterinary Sciences, Autonomous University of Barcelona, Bellaterra (Cerdanyola del Vallès), Spain*

1. Introduction

Aquaporins (AQP) are a family of transmembrane proteins that ease the transport of water and small solutes, including orthodox AQPs, aquaglyceroporins (GLPs) and superAQPs. The role of AQPs in volume regulation and osmoregulation of sperm cells, and the drastic osmotic stress occurring during cryopreservation explain the relationship between AQP levels and sperm cryotolerance. The aim of this study was to elucidate the functional relevance of different groups of AQPs during mammalian sperm cryopreservation, using two animal models (pigs and horses).

2. Methodology

A total of 27 pools from 42 boar ejaculates and 12 stallion ejaculates were used. After dilution in cryopreservation media, sperm samples were split into different subfractions: the control, and the treatments with different AQP inhibitors (1,3-propanediol, PDO; acetazolamide, AC; and phloretin, PHL). Samples were cryopreserved with a controlled-rate freezer and thawed in a water bath. Sperm quality and function were assessed in fresh samples, and after 30 and 240 min (boar), or after 10 and 120 min (stallion) of thawing. Total (TMOT) and progressive motility (PMOT) were assessed through a computer-assisted sperm analysis system, and flow cytometry analyses were performed to assess viability, acrosome integrity, membrane lipid disorder, mitochondrial membrane potential (MMP), and intracellular levels of calcium, peroxides and superoxides.

3. Results

Stallion spermatozoa treated with PDO presented higher percentages of TMOT, PMOT, spermatozoa with an intact acrosome, and spermatozoa with high levels of calcium than the control ($P<0.05$). In addition, both boar and stallion PDO-treated samples showed higher viability and MMP, and lower membrane lipid disorder ($P<0.05$). Boar spermatozoa treated with PDO showed higher levels of peroxides and superoxides than the control ($P<0.05$).

In contrast, stallion samples treated with PHL showed lower TMOT, PMOT, sperm viability, acrosome integrity, and higher MMP and intracellular calcium levels than the control ($P<0.05$). In boar samples, these effects were restricted to the highest concentration of PHL ($P<0.05$). Finally, the effects of AC were neither consistent nor concentration-dependent.

4. Discussion

The effects of PDO suggest that instead of being an AQP inhibitor, it acts as a cryoprotective agent, with more apparent effects on stallion than on boar sperm. While the effects of PHL underpin the

relevance of GLPs for mammalian sperm cryotolerance, those of AC suggest that orthodox AQPs are either absent in boar and stallion sperm or irrelevant during cryopreservation.

5. Conclusion

The inhibition of different groups of AQPs suggests that GLPs are more relevant than orthodox AQPs in mammalian sperm cryopreservation.

Keywords: aquaporins, mammalian, cryopreservation, sperm

6. References

Bonilla-Correal, Sebastián.; Noto, Federico; Garcia-Bonavila, Estela; Rodríguez-Gil, Joan E.; Yeste, Marc; Miró, Jordi (2017). "First evidence for the presence of aquaporins in stallion sperm". *Reprod. Domest. Anim.*, vol. 52, no. S4, p. 61–64.

Gao, Junwei; Wang, Xiaohua; Chang, Yongjie; Zhang, Jianzhao; Song, Qianliu; Yu, Heming; Li, Xuejun (2006). "Acetazolamide inhibits osmotic water permeability by interaction with aquaporin-1". *Anal. Biochem.*, vol. 350, no. 2, p. 165–170.

Prieto-Martínez, Noelia; Morató, Roser; Vilagran, Ingrid; Rodríguez-Gil, Joan E.; Bonet, Sergi; Yeste, Marc (2015). "Aquaporins in boar spermatozoa. Part II: detection and localisation of aquaglyceroporin 3". *Reprod. Fertil. Dev.*, vol. 20, no.4, p. 703–711.

Prieto-Martínez, Noelia; Vilagran, Ingrid; Morató, Roser; Rodríguez-Gil, Joan E.; Yeste, Marc; Bonet, Sergi (2014). "Aquaporins 7 and 11 in boar spermatozoa: detection, localisation and relationship with sperm quality". *Reprod. Fertil. Dev.*, 28 (6), p. 663–672.

Tanimura, Yukihiro; Hiroaki, Yoko; Fujiyoshi, Yoshinori (2009). "Acetazolamide reversibly inhibits water conduction by aquaporin-4". *J. Struct. Biol.*, vol. 166, no. 1, p. 16–21.

Wacker, Sören J.; Aponte-Santamaría, Camilo; Kjellbom, Per; Nielsen, Soren; De Groot, Bert L.; Rützler, Michael (2013). "The identification of novel, high affinity AQP9 inhibitors in an intracellular binding site". *Mol. Membr. Biol.*, vol. 30, no. 3, p. 246–260.

Yeste, Marc; Morató, Roser; Rodríguez-Gil, Joan E.; Bonet, Sergi; Prieto-Martínez, Noelia (2017). "Aquaporins in the male reproductive tract and sperm: Functional implications and cryobiology". *Reprod. Domest. Anim.*, vol. 52, no. S4, p. 12–27.

Yu, Lili; Rodriguez, Roberto A.; Chen, L. Laurie; Chen, Liao Y.; Perry, George, McHardy, Stanton F.; Yeh, Chih-Ko (2016). "1,3-Propanediol binds deep inside the channel to inhibit water permeation through aquaporins". *Protein Sci.*, vol. 25, *no.* 2, p. 433–441.