

[10.1071/RD23091](https://doi.org/10.1071/RD23091)

Reproduction, Fertility and Development

Supplementary Material

Immunolocalisation of aquaporins 3, 7, 9 and 10 in the epididymis of three wild ruminant species (Iberian ibex, mouflon and chamois) and sperm cryoresistance

Belen Martinez-Madrid^{A,}, Carlos Martínez-Cáceres^B, Belén Pequeño^C, Cristina Castaño^C, Adolfo Toledano-Díaz^C, Paula Bóveda^C, Paloma Prieto^D, Manuel Alvarez-Rodriguez^C, Heriberto Rodriguez-Martinez^E, and Julián Santiago-Moreno^C*

^ADepartment of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid 28040, Spain.

^BPathology core, Biomedical Research Institute of Murcia Pascual Parrilla (IMIB), Ctra. Buenavista s/n, El Palmar, Murcia 30120, Spain.

^CDepartment of Animal Reproduction, National Institute for Agricultural and Food Research and Technology, Spanish Scientific Research Council (INIA-CSIC), Avda. Puerta de Hierro km 5.9, Madrid 28040, Spain.

^DConsejería de Sostenibilidad, Medio Ambiente y Economía Azul, Junta de Andalucía, Jaén, Spain.

^EDepartment of Biomedical and Clinical Sciences (BKV), Obstetrics and Gynecology, Linköping University, Linköping, Sweden.

*Correspondence to: Belen Martinez-Madrid Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid 28040, Spain Email: belmart@ucm.es

Fig. S1. Positive controls for aquaporin 3, 7, 9, and 10 immunohistochemistry. (a) Mouse kidney (for AQP3). (b) Rat kidney (for AQP7). (c) Rat liver (for AQP9). (d) Human jejunum (for AQP10). 400X. Bar = 50 μ m.

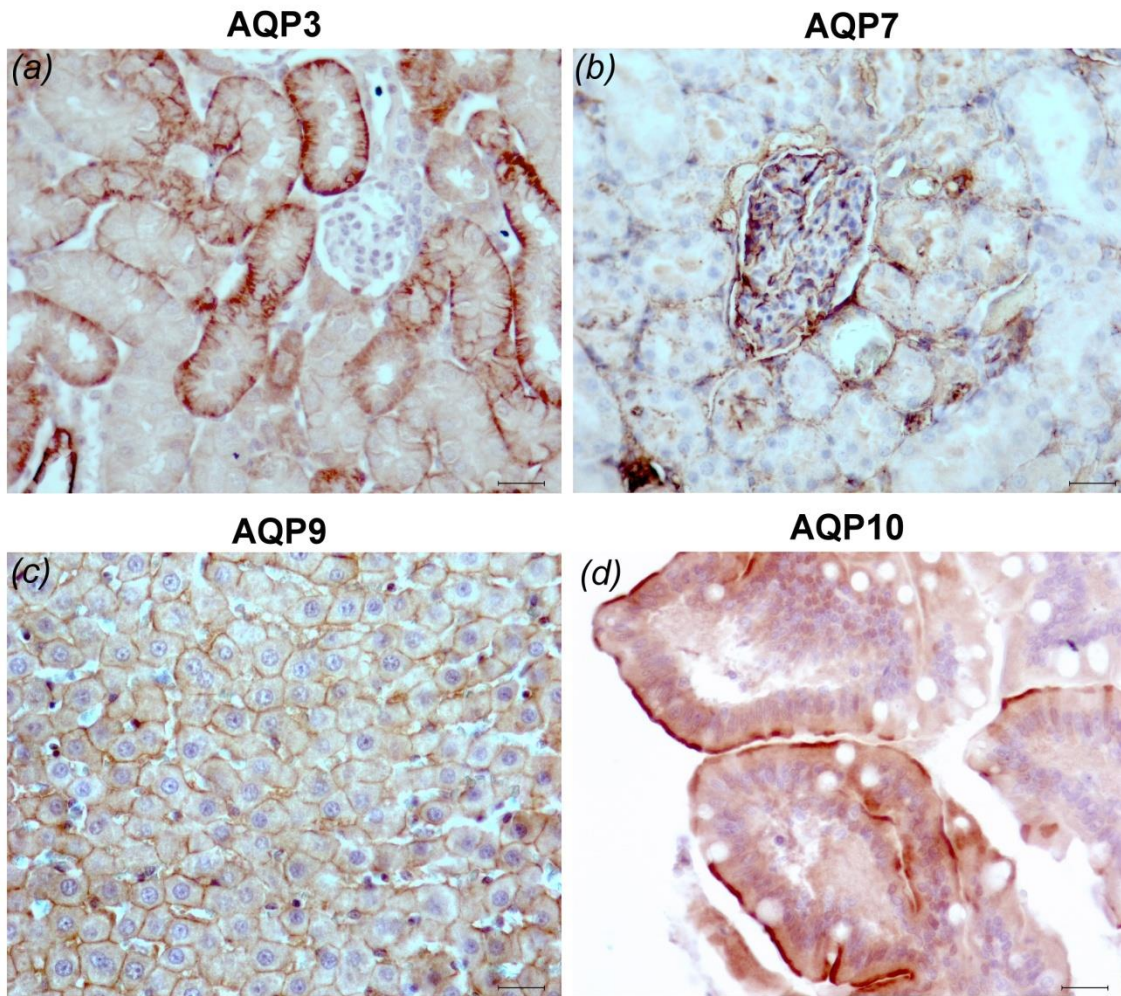


Fig. S2. Negative controls for aquaporin 3, 7, 9, and 10 immunohistochemistry of Iberian ibex, mouflon, and chamois epididymis. (a-c) Caput, (d-f) corpus, (g-i) and cauda regions of Iberian ibex, mouflon, and chamois epididymis, respectively. 400X. Bar = 50 μ m.

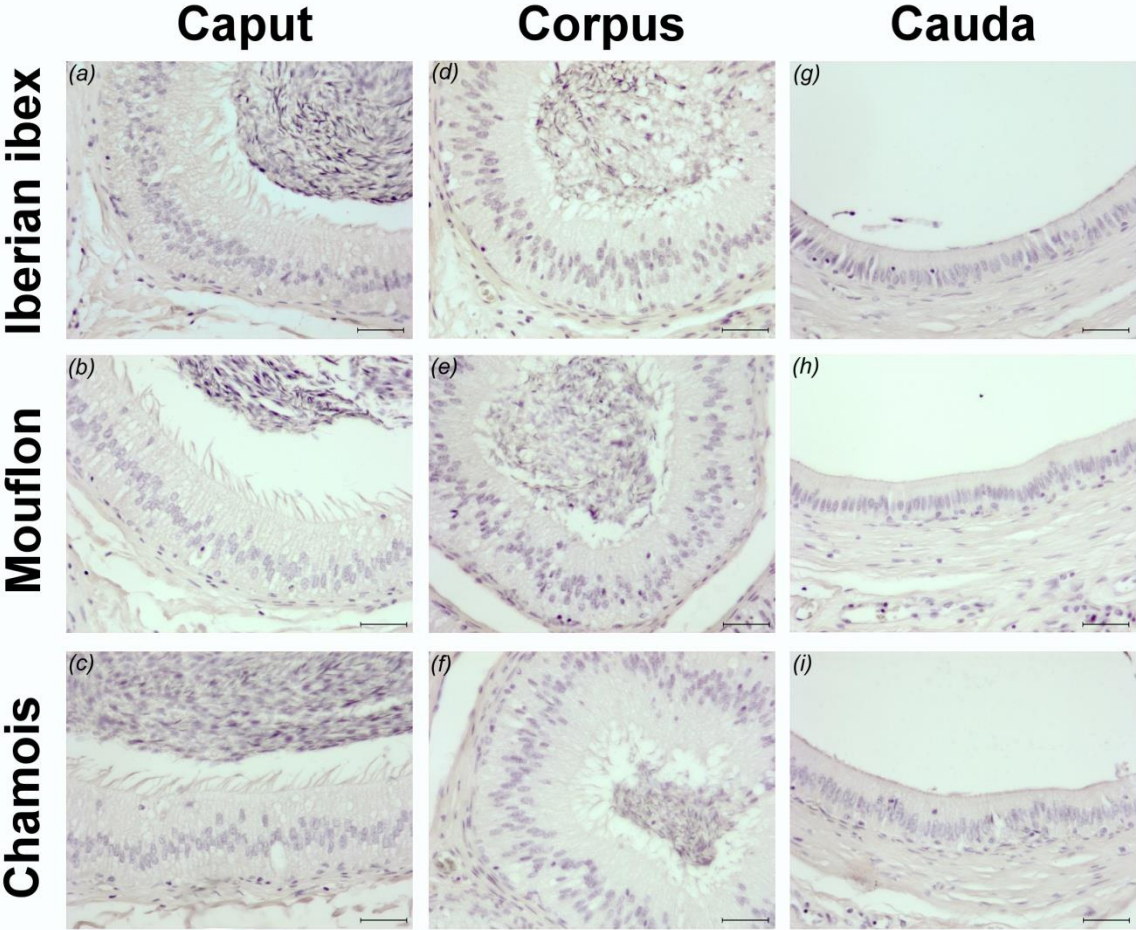


Fig. S3. Peptide-blocking experiments to test the specificity of aquaporin 3, 7, and 9 antibodies in caput, corpus, and cauda region of Iberian ibex epididymis and ovine kidney. For aquaporin 3, 7, and 9, in the upper files (a-d, i-l, q-t), the positive immunolabeling after incubating with the primary antibody, and in the lower files (e-h, m-p, u-x), the lack of immunolabeling resulting from incubation also with the respective aquaporin blocking peptide. 630X. Bar = 50 μ m.

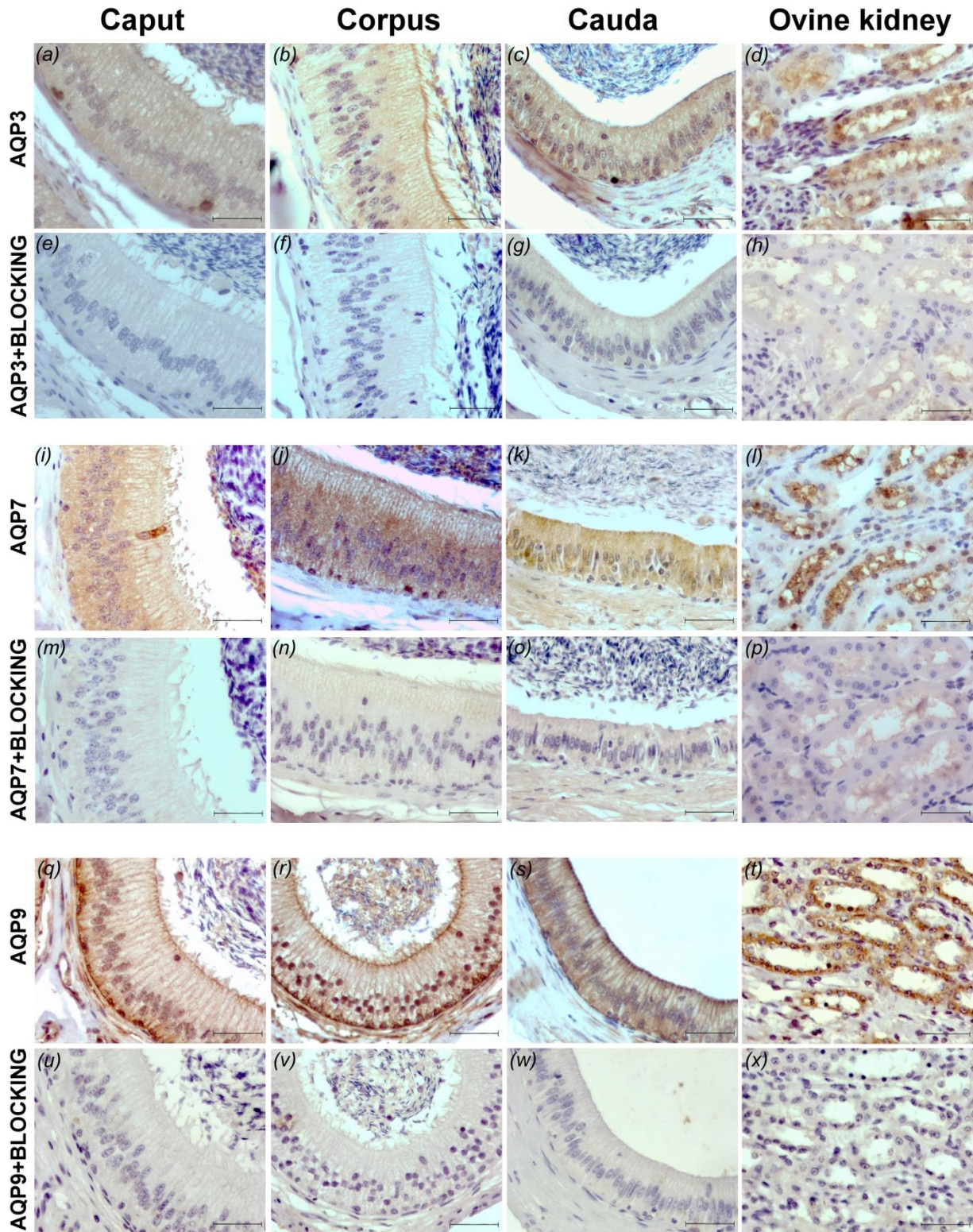


Fig. S4. Detail of aquaporin 3, 7, 9, and 10 immunolabeling in caput region of Iberian ibex, mouflon, and chamois epididymis. 630X. Bar = 50 μ m.

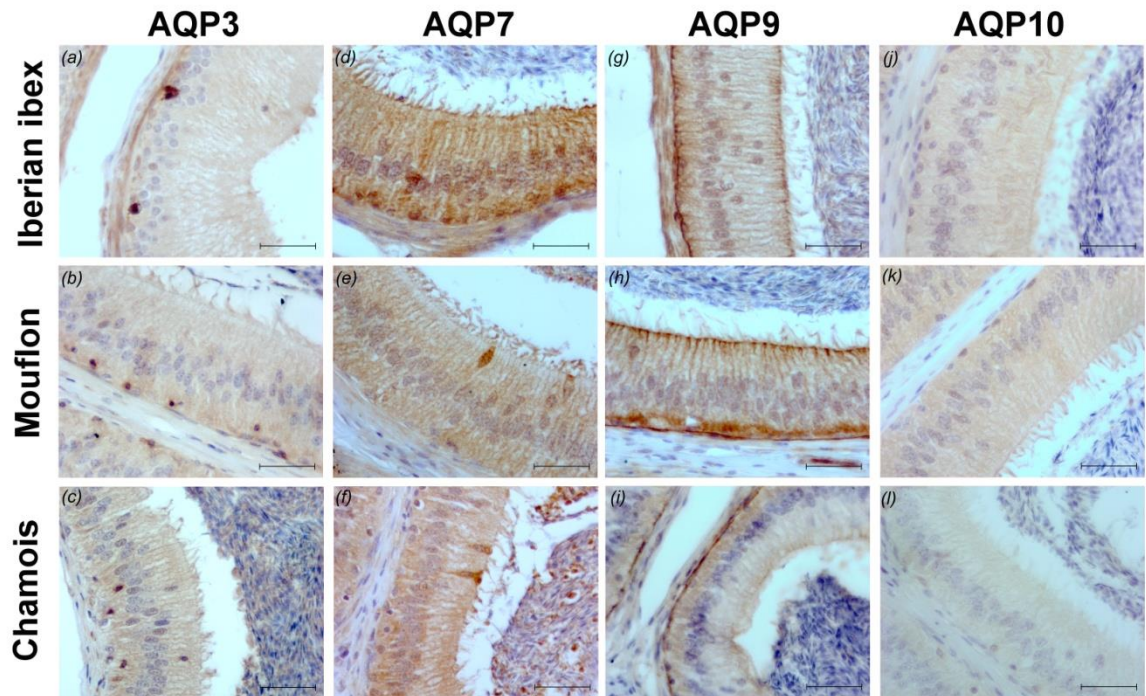


Fig. S5. Detail of aquaporin 3, 7, 9, and 10 immunolabeling in corpus region of Iberian ibex, mouflon, and chamois epididymis. 630X. Bar = 50 μ m.

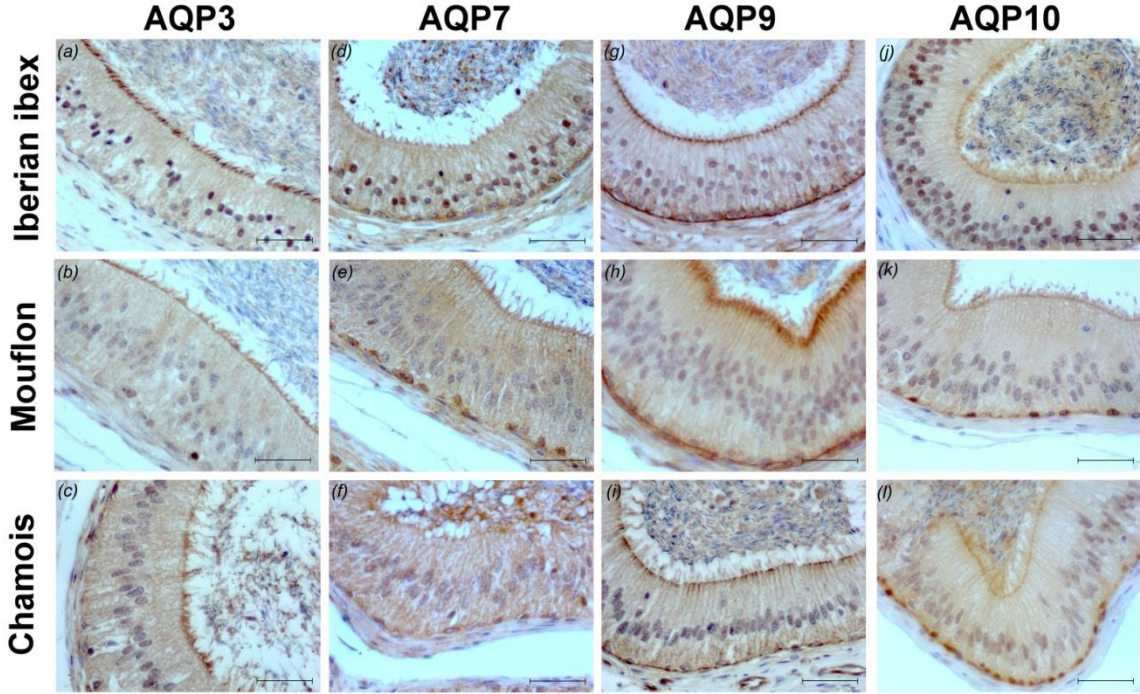


Fig. S6. Detail of aquaporin 3, 7, 9, and 10 immunolabeling in cauda region of Iberian ibex, mouflon, and chamois epididymis. 630X. Bar = 50 μ m.

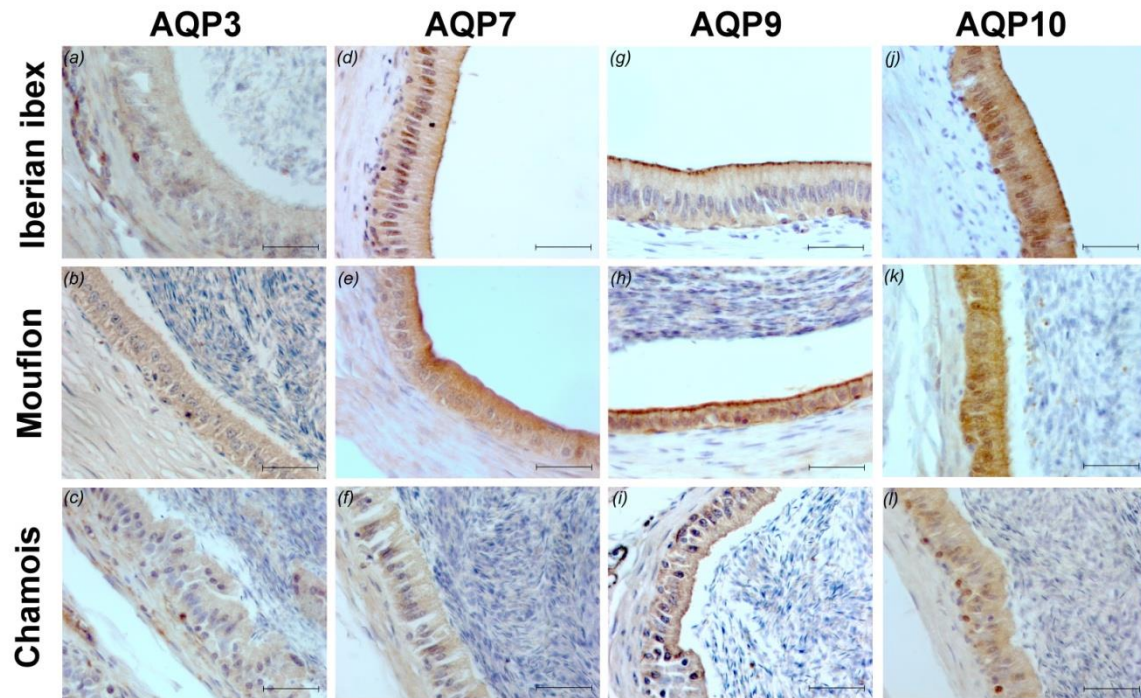


Fig. S7. Illustrative images of the apical blebs immunolabeling in the caput, corpus, and cauda regions of the epididymal epithelium. The images show the aquaporin 9 immunolabeling in the lumen of caput (a), corpus (b), and cauda (c) epididymis of Iberian ibex as a representative example for all the studied AQPs and wild ruminant species. 630X. Bar = 50 μ m.

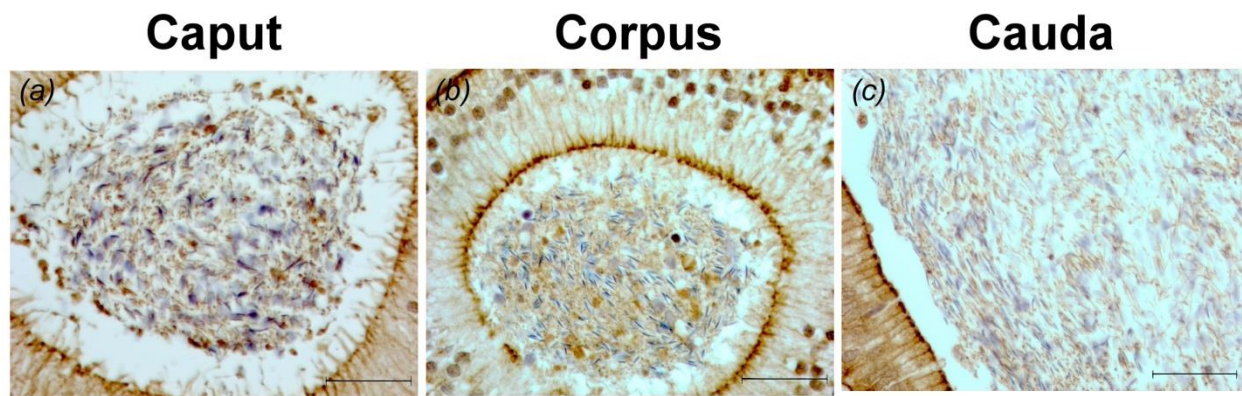


Table S1. Immunolabeling of AQP3, AQP7, AQP9, and AQP10 in the apical blebs of Iberian ibex, mouflon, and chamois epididymal epithelium.

Epididymal region	Species	AQP3	AQP7	AQP9	AQP10
Caput	Ibex	-	+	+	+
	Mouflon	-	+	+	+
	Chamois	+	+	+	+
Corpus	Ibex	+	+	+	+
	Mouflon	+	+	+	+
	Chamois	+	+	+	+
Cauda	Ibex	-	+	+	-
	Mouflon	-	+	+	-
	Chamois	-	+	+	-