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# Effects of Putative Epididymal Osmolytes on Sperm Volume Regulation of Fertile and Infertile *c-ros* Transgenic Mice

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Volume regulation by spermatozoa has been demonstrated to be crucial in both mice and men for transport in the female tract. In order to determine the nature of osmolytes used by spermatozoa, they were released from the cauda epididymis of fertile *c-ros* heterozygous mice into incubation medium of uterine osmolality (representing an osmotic challenge), containing increasing concentrations of compounds that are major epididymal fluid components and known osmolytes in somatic cells. This should nullify the concentration gradients for osmolytes that mediate volume regulation, prevent osmolyte efflux, and lead to swelling. Of the osmolytes tested, K<sup>+</sup> caused the most rapid and extensive volume increases; glutamate, taurine, L-carnitine, and myo-inositol also were effective, but glycerophosphocholine was not. Such effects were not observed in cauda sperm from the infertile knockout mice, demonstrating a defect in normal volume regulation. K<sup>+</sup> concentrations in cauda epididymal fluid were 21 mM higher in the knockout than the heterozygous mice, but no differences were found in caudal fluid glutamate, carnitine, or myo-inositol. The carnitine content of cauda sperm from knockout males was not different from that of fertile males, but lower amounts of glutamate and inositol were found that could explain the poor volume regulation. In heterozygous mice, cauda but not caput sperm responded to the K<sup>+</sup> channel blocker quinine by swelling, demonstrating development of volume regulation during epididymal transit, whereas knockout cauda sperm showed no response, as with the osmolytes. Major epididymal secretions could serve as osmolytes in murine spermatozoa for volume regulation in response to physiological osmotic challenge in the normal fertile mice; the reduced sperm content of inositol and glutamate in the *c-ros* knockout mice might reflect maturational abnormalities in volume regulation.

Key words: Sperm swelling, regulatory volume decrease, quinine, organic osmolytes, infertility

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