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Measurement of Volume Changes in Mouse Spermatozoa Using an Electronic Sizing Analyzer and a Flow Cytometer: Validation and Application to an Infertile Mouse Model

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The importance of sperm volume has recently been highlighted in a knockout mouse model in which infertility was caused by defects in volume reguiation, which led to sperm transport failure in the female tract. Inhibition of volume regulation by human sperm, resulting in failure of penetration of cervical mucus in vitro, has also been reported. The present work aims to establish a sensitive and convenient method for monitoring changes in sperm volume for functional studies. Mature murine sperm obtained from the cauda epididymidis were analyzed by flow cytometry for their forward and side (90°C) scatter of a 488-nm excitation wavelength laser, and the data were compared with volumes measured by electronic sizing using a Coulter counter. Changes in cell volume were induced by releasing or diluting sperm into culture media of various osmolalities (208-520 mmol/kg). Forward scatter signal (FSS) intensity correlated well with volume measurement obtained by a Coulter counter (R =.83; P <.001), confirming that FSS reflects Coulter counter findings as for somatic cells. Sperm swelling was also induced by the presence of quinine, a wide-spectrum channel blocker, in a medium of 330 mmol/kg, which is similar to the osmolality of uterine fluid. The effect of quinine on sperm volume was more obvious when analyzed by flow cytometry than by electronic sizing. This effect was even more marked after dead sperm identified by fluorescent dye were eliminated from analysis using flow cytometry. Swelling was characterized by an increase in forward scatter and side scatter, generating a subpopulation of sperm that correlated well (R =.79; P <.0001) with the population of sperm exhibiting an angulation of the tail, which is a morphological manifestation of swollen murine sperm. Flow cytometric analysis revealed that infertile sperm released from the cauda epididymidis of c-*ros* knockout mice were significantly larger than those of fertile sperm from heterozygous mice. This finding directly substantiates the suggestion that infertile sperm are defective in their volume regulation. Laser scatter analysis of viable murine sperm by flow cytometry offers a convenient and sensitive method for the study of sperm volume regulation.

Key words: Coulter counter, c-*ros* knockout mice, hypo-osmotic swelling, regulatory volume decrease, sperm function

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