Alan Young

Major: Chemical Engineering University: University of Michigan-Ann Arbor Faculty Mentor: Dr. John Critser Mentor Department: Veterinary Pathobiology Funded by: NSF-REU Biosystems Modeling

Bovine oocyte permeability in 1, 2propanediol

Alan Young, Steven Mullen and John Critser

Cryopreservation of oocytes is of great importance both in medical research and in the agricultural industry. Cryopreservation of oocytes allows for future breeding of selected genetic lines of animals. Effective cryopreservation of bovine oocytes has drawn considerable attention due to its application in the agricultural industry. The reproductive cells of genetic lines of cattle can now be cryopreserved and used in the future to breed superior cattle through in vitro fertilization (IVF). Currently, the preservation of bovine oocytes and other cells has had marginal success due to damage sustained to the cell during freezing and thawing due to volume fluctuations (Mazur et al; Experimental Cell Research 71(1972) 345-355). The Kedem and Katchalsky model (Biochem Biophys Acta, 1958, 27:229-246) can be utilized to model changes in cellular volume during freezing and thawing in cryoprotectant solutions. This model takes into account the specific plasma membrane permeability of the cell that is exposed to a particular solution. The purpose of this study was to determine the hydraulic conductivity, Lp, and the permeability coefficient for the cryoprotective agent, 1, 2propandiol (PrOH), PCPA, for bovine oocytes. The activation energies of each of these parameters can be determined under the assumption that the plasma membrane permeability parameters follow an Arrhenius relationship. Experimental trials were conducted at temperatures of 30°C, 20°C, 10°C, and 4° C. In order to study the response of a single bovine oocyte to 1.5 M 1, 2propanediol, a micro-pipette holding device (Gao et al.; Biophysics J, 71:443-450) was used to immobilize the oocyte in a small drop of TL-Hepes media. The oocyte was then abruptly exposed to 1.5 M PrOH media. The volume change of the oocyte (dv/dt) was recorded with a digital camera that was mounted to an inverted light microscope. The area of the cell in each image was calculated with a Fovea Pro Software plug-in to Adobe Photoshop. The volume of the cell was calculated from the calculated area, assuming that the cell was spherical. The constants, Lp and PCPA, were numerically approximated assuming that the cellular volume dynamics followed the Kedem and Katchalsky model. It was found that the oocytes underwent osmotically driven volume changes upon exposure to the cryoprotectant, 1, 2-propanediol. The bovine oocytes contracted more rapidly at higher temperatures. The oocytes also regained their isosmotic volume faster at higher temperatures. The permeability parameters found in this study along with there activation energies will be used in the future to develop an optimal cryopreservation protocol for bovine oocytes through computer-based modeling.