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Membrane permeability of Bovine Ooctyes to Propylene Glycol and the application to the improvement of Cryopreservation

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In this study, the goal was to determine the permeability parameters of bovine oocytes for water (Lp) and Propylene Glycol (PPG) at temperatures of 30, 20, 10, and 5°C. By determining permeability parameters, we can model cell volume changes during addition and removal of cryoprotectants to determine a method that will prevent osmotic damage to the cells. Individual oocytes were held stationary by a holding pipette in a Petri dish on a Nikon inverted microscope. The oocytes were initially equilibrated in propylene glycol (PG) and 0.1M Sucrose for 20 minutes and then a solution of TL-Hepes with 0.1M Sucrose was added to a drop of 1.5M PG containing the oocyte. The specific initial concentration of PG and volumes of added solutions were modified for each temperature. Then digital images were captured on a regular time scale using a Spot RT Cooled CCD Digital camera in order to record shrinking and swelling. Morphometrical analysis was then performed on each image using Adobe Photoshop to measure the radius of each oocyte at the various time points during the volume excursions. Using Microsoft Excel, we were able to fit the experimental data to a best fit curve of a theoretical model for volume change, which allowed the determination of the values of Lp and PPG. These values were used to model the cell volume changes using MLAB (Civilized Software, Inc., Bethesda, MD) to developing optimized addition and removal procedures for 3.0M CPA that would minimize potential damage of the oocyte due to shrinking and swelling, and toxicity effects of the CPA due to excessive exposure. Currently, our results for the mean values of the permeability parameters Lp and PPG at 20°C are 0.3 ± 0.03 µm-min.atm and 15 ± 7.2 µm/min, respectively (mean ± SD, n=2). Further data acquisition and analysis is in progress.