CDB SEMINAR

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The current paradigm of mammalian oocyte cryopreservation, and a proposed alternative

The mammalian oocyte has played a prominent role in the development of the science of Cryobiology. Following up on the success of spermatozoa cryopreservation in the late 1940's, attempts to cryopreserve rabbit oocytes using glycerol as the cryoprotectant were undertaken. Unfortunately, the outcomes of these early studies were rather disappointing. The recalcitrance of mammalian oocytes to cryopreservation is highlighted by the fact that it took nearly 20 more years before the first live births were reported using frozen and thawed mouse oocytes. With hindsight, some of the reasons for the early failures can be understood. One feature of mature oocytes that makes them particularly susceptible to cryoinjury is their large volume (on the order of 3 to 10 x 10^5 cubic micrometers, depending upon the species) and spherical shape. Their low surface area-to-volume ratio, coupled with the fact that vitrification appears to be necessary to achieve cryopreservation for a high proportion of oocytes from many species, makes the cells susceptible to osmotic and chemical damage during cryoprotectant addition and removal. Oocytes from many mammals are also very sensitive to cooling. Some of the factors responsible for this sensitivity include the amount of cytoplasmic lipids and damage to the meiotic spindle. In 1996, it was shown that the potential of bovine oocytes to develop in culture was affected after cooling for as little as 5 seconds. Mouse oocytes are more tolerant of cooling, which may explain why oocytes from mice were the first to be successfully cryopreserved as measured by the retention of their full developmental potential. It was the demonstration of the extreme sensitivity of bovine oocytes to cooling that resulted in a paradigm shift in the field; at the present time nearly all studies directed at improving cryopreservation of oocytes from cows and pigs utilize "ultra-rapid" vitrification methods in an effort to out-race chilling injury. However, chilling injury is only one of many possible sources of injury to cells during a routine cryopreservation procedure, and the time for which it takes a solution in a standard freezing straw to move through the zone of chilling injury upon plunging into liquid nitrogen is only a fraction of a second. Other sources of injury include toxicities associated with high concentrations of cryoprotectants (both osmotic and chemical). Previously, it has been shown that modification of a CPA addition and removal procedure to include a longer stepwise procedure can significantly increase the proportion of bovine oocytes that maintain developmental viability when vitrified using a standard freezing straw. Computer modeling designed to identify an optimal method for vitrifying bovine oocytes in a solution containing ethylene glycol and sucrose as cryoprotectants gave results very similar to those developed empirically. It is proposed that vitrification with the so-called "ultra-rapid" cooling methods are unnecessary if a more fundamental approach to the design of improved oocyte vitrification methods is taken.

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