Letters to the Editor

Use of amiloride to reduce osmotic stress injury of mouse oocytes during cryopreservation

Dear Sir,

We read with interest the recent paper by Inagaki et al. (1996) detailing the use of amiloride (an Na+/H+ exchange inhibitor) as an additive to improve recovery of mouse oocytes after cryopreservation. The drug was found to inhibit cortical granule exocytosis, second polar body extrusion and pronuclear formation which occurred in mouse oocytes parthenogenetically activated after osmotic stress induced by exposure to dimethyl sulphoxide (DMSO) as cryoprotectant. The authors described a reduced recovery of degenerate oocytes (23 versus 39% without drug) and higher fertilization (24 versus 18% without drug) using amiloride in the cryopreservation protocol, and recommended that the drug may be a useful way to increase the survival of cryopreserved oocytes.

The protocol used by Inagaki et al. for addition and removal of DMSO at room temperature in itself induced a high rate (39%) of degeneration in thawed oocytes. It is increasingly recognized that several variables, including time and temperature of cryoprotectant exposure, cooling profiles during cryopreservation and use of an osmotic agent during dilution, can be manipulated to yield good recoveries of oocytes after cryopreservation (Bernard and Fuller, 1996). For example, George et al. (1994) employed 1.5 M DMSO as cryoprotectant at an exposure temperature of 4°C with slow cooling to –35°C before plunging in liquid nitrogen to yield recoveries of 79% and fertilization of 63%. We have also reported a method for exposing mouse (and human) oocytes to 1 M DMSO at 4°C and slow cooling to –70°C before transfer to –196°C with recoveries of 88% and fertilization of 63% (Hunter et al., 1991). Choice of rapid cooling or vitrification techniques necessitates different, but equally stringent, control of conditions during the various steps in each protocol (Wood et al., 1993; Cooper et al., 1996; O’Neil et al., 1997). All these methods still require considerable further investigation and refinement before they can be reliably used in the clinical setting. However, we feel that these approaches to optimize the cryobiological variables should be exhausted before moving to the use of additional pharmacological agents which themselves would require careful investigation for other, unwanted effects on the fertilization process or subsequent embryonic development after oocyte cryopreservation.

References


© European Society for Human Reproduction and Embryology

Sharon Paynter, Angela Cooper, Louise O’Neil and Barry Fuller

Department of Obstetrics and Gynaecology,
University of Wales College of Medicine,
Heath Park, Cardiff CF4 4XN, UK

Dear Sir,

We thank Drs Paynter, Cooper, O’Neil and Fuller for their advice. We have never used 5-[(N,N dimethyl)-amiloride clinically for human oocytes. We agree with them that the side-effects of this medicine on embryonic development should be investigated in detail before clinical use, and that many other methods of oocyte cryopreservation for the concentration of DMSO, temperature and method of cooling could be tried before medicines are used.

Noboru Inagaki

Department of Obstetrics and Gynaecology,
School of Medicine, Keio University,
35 Shinanomachi, Shinjuku-ku, Tokyo 106, Japan