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What Can We Expect from Thawing Gametes?

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Cryopreservation of cells and tissues has been applied for many decades with variable success. In the case of cell lines, the survival of a few percent of frozen cells will allow the re-establishment of cell cultures, particularly for robust cell types. Success of sperm cryopreservation is also based on the presence of many sperm cells (usually 4 to 25 x 106 sperm in each insemination straw) and the survival of a reasonable percentage of these sperm (10-50%). The demand for very high survival rates for human embryos has resulted in more refined and defined freezing methods involving very slow cooling rates during the ice formation phase (equilibration cooling). This enables embryo survival rates to be higher than 50% (often approaching 80%) and the application of embryo cryopreservation in clinical IVF. Implantation rates of surviving embryos varies from 8 to 20% and may not be very different to that of non-frozen embryos (1,2). Under these circumstances there is not much inducement to adopt new cryopreservation techniques such as vitrification for human embryos, although it has been reported to be successful. High concentrations of ethylene glycol (40%) and the polymer Ficoll (18%) together with 0.3 M (10%) sucrose have been used for cryopreservation of early cleavage stage human embryos, and the production of a set of healthy twins (1 of 18 patients transferred embryos) (3). Other cell types have also been vitrified using high concentrations of permeating cryoprotectants including human cord blood hematopoietic cells (4), tissue containing nerve segments (from rat hind leg) (5), liver slices (6), and established cell culture lines (osteosarcoma and malignant fibrous histosarcoma) (7). However, reproducibility of high cryosurvival rates with vitrification remains a problem for many cell types. Langer et al. (8) found low cryosurvival after vitrification of human pancreatic islet cells compared with conventional freezing. Human corneal endothelial tolerance to the high levels of permeating cryoprotectants needed for vitrification is also low (9).

Success rates for cryopreserved human oocytes have been extremely disappointing and far below survival rates that are required for clinical application. The most advanced data for the conventional slow-cooling of human oocytes has been reported by Dr. E. Porcu (10) from the University of Bologna. Mature oocytes were frozen using a minor modification of the technique used for human embryos involving the permeating cryoprotectant 1,2-propanediol (11). Of 753 thawed oocytes, 7 babies (6 births) were born which is an overall success rate of less than 1%. This is not significantly different to that achieved with the original slow cooling techniques reported for human oocytes (12) that resulted in a small number of births

in the late 1980's (13, 14). The only advance in methodology is the use of intracytoplasmic sperm injection (ICSI) to achieve fertilization in thawed oocytes because it is argued that this overcomes the hardening of the zona pellucida that may be caused by premature cortical granule release during freezing of oocytes (15). The large scale studies of Porcu et al. (10) do not appear to show any substantial benefit of ICSI on the development of thawed oocytes and there are no controlled studies to support the increased developmental competence of thawed oocytes fertilized by ICSI rather than conventional IVF. Tucker et al. (16) also reported the outcomes of thawing 334 human oocytes frozen by basically the same method as Porcu et al. (10). Only 70 (21%) oocytes survived thawing and 25 developed to transferable embryos. Three viable pregnancies involving four advanced fetuses were established after embryo transfer to recipient patients (an overall success rate of around 1%). It has also been shown recently that sodium salts are damaging to mouse oocytes during these conventional slow cooling methods for freezing because sodium is the major ion involved in the solution effects which cause cryoinjury to cell membranes during dehydration and rehydration (17). Substitution of sodium with choline in the cryopreservation medium significantly improved oocyte survival and developmental rates after freezing and thawing. An attempt to vitrify human oocytes in the original vitrification solution used by Rall and Fahy (18), which is a mixture of the permeating cryoprotectants (dimethyl sulphoxide, propanediol and polyethylene glycol plus acetamide), resulted in 9/20 (45%) fertilized oocytes but no further cleavage and development (19).

Recently we have shown that human oocytes can be vitrified successfully in low toxicity solutions of ethylene glycol, sucrose and human serum albumin. In the initial preliminary studies (20) of a small number of thawed oocytes, 67% survived vitrification intact, and 80% of these surviving oocytes fertilized and developed to 6- to 10-cell embryos by day 3. When three embryos were transferred to three patients, a single pregnancy and birth of a normal child resulted. Further studies have now been undertaken at the SISMER IVF Clinic, Bologna and further pregnancies have been achieved. Human oocytes have also been vitrified in 5.5 M ethylene glycol and 1 M sucrose on microscope copper grids (21). Oocytes survived vitrification and fertilized at relatively high rates (38-71%, depending on group treatment). The embryos developed to 4- to 8-cells and to blastocysts in some treatment groups. These new data will be discussed and the potential applications explored in more detail.

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