CASE REPORT

Birth following vitrification of a small number of human oocytes

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We report the birth of a healthy baby girl at 37 weeks gestation to a 47 year old recipient, after vitrification of mature oocytes from four in-vitro fertilization (IVF) patients. A total of 17 oocytes was vitrified in 1–2 µl of ethylene glycol (40%) and 0.6 mol/l sucrose (20.54%) in open pulled straws. Eleven oocytes survived after vitrification and five pronuclear zygotes were obtained after intracytoplasmic sperm injection (ICSI). Three embryos were transferred to three patients, two of whom were the original oocyte donors and pregnancy was not established. The third embryo was donated to a 47 year old infertile woman after preimplantation diagnosis had confirmed euploidy for chromosomes X, 13, 14, 15, 16, 18, 21 and 22. The successfully completed pregnancy is encouraging for further research to explore the potential benefits of vitrification for the cryopreservation of human oocytes, given the relatively low success of conventional freezing of human oocytes by slow cooling methods. Key words: birth after freezing/cryopreservation/oocyte/oocyte cryosurvival/vitrification

Introduction

The cryopreservation of human oocytes has remained a difficult and inefficient procedure despite considerable effort to improve the original methods described by Trounson (1986). The introduction of 1,2 propanediol as an alternative to dimethyl sulfoxide as the penetrating cryoprotectant and intracytoplasmic sperm injection (ICSI) to overcome fertilization failure after cryopreservation has had little effect on the overall success rates (Porcu et al., 1997). Of more than 709 oocytes thawed, six babies were reported (Porcu et al., 1998b), which is less than 1% of all thawed oocytes. Similarly, Tucker et al. (1998) reported around 1–2% of more than 400 thawed oocytes developing to term using a very similar technique. These data are little different from those which resulted in the first pregnancies from frozen oocytes (Chen, 1986; Van Uem et al., 1987). Smaller studies, such as that of 10 donor oocytes frozen (de Fried et al., 1998), which resulted in a single birth, have been encouraging but the methods were the same as the earlier, larger study (Tucker et al., 1998). We have been exploring the use of vitrification using very rapid cooling for cryopreservation of human oocytes and report the birth of a normal child resulting from the vitrification and transfer of a small number of human oocytes.

Case report

Four patients of mean age 32.5 years (range 27–35) attending the SISMER in-vitro fertilization (IVF) clinic (Bologna, Italy) agreed to have their surplus oocytes vitrified with the intention of reducing the number of surplus zygotes and embryos that needed to be frozen in the particular cycle of treatment. A total of 17 oocytes from the four patients was vitrified, 13 were intended to be used by the patients to form embryos for their own use and four were donated to recipients seeking donor oocytes.

Oocytes were recovered after conventional ovarian stimulation for IVF as previously described (Ferraretti et al., 1996). Two to seven of the mature metaphase II oocytes from the four patients were randomly allocated to the vitrification study. Within 4 h of aspiration from follicles, the oocytes were denuded of cumulus cells by a brief exposure to hyaluronidase enzyme (Hyase®; Scandinavian IVF Sciences, Göteborg, Sweden). Cumulus denuded mature metaphase II oocytes were transferred to a 10% (v/v) ethylene glycol (Sigma, St Louis, MO, USA) solution in phosphate buffered saline (PBS) (Gibco BRL, Paisley, Renfrewshire, UK) containing 10 mg/ml human serum albumin (HSA) (Advanced Reproductive Technologies Inc., San Clemente, CA, USA) for 40 s. Oocytes were then transferred to 20% ethylene glycol (v/v) in PBS + 10 mg/ml HSA for 30 s and finally to 40% ethylene glycol (v/v) and 20.54% (w/v) (0.6 mol/l) sucrose (Analar®; BDH Laboratory Supplies, Poole, Dorset, UK) in PBS + 10 mg/ml HSA for a further 60 s. All the procedures were carried out on a warm stage at 37°C. The oocytes were then drawn into a finely drawn plastic straw by capillary action (Vajta et al., 1998) and the open pulled straw (OPS) rapidly cooled to −196°C (at about 20 000°C/min) by direct transfer to liquid nitrogen. The OPS straws were drawn from heated plastic 0.25 ml insemination straws as described by Vajta et al. (1998). Oocytes were then transferred into a 13.69% ethylene glycol (v/v) in PBS + 10 mg/ml HSA for 30 s and finally to 40% ethylene glycol (v/v) and 20.54% (w/v) (0.6 mol/l) sucrose (Analar®; BDH Laboratory Supplies, Poole, Dorset, UK) in PBS + 10 mg/ml HSA for a further 60 s. All the procedures were carried out on a warm stage at 37°C. The oocytes were then drawn into a finely drawn plastic straw by capillary action (Vajta et al., 1998) and the open pulled straw (OPS) rapidly cooled to −196°C (at about 20 000°C/min) by direct transfer to liquid nitrogen. The OPS straws were drawn from heated plastic 0.25 ml insemination straws as described by Vajta et al. (1998). Oocytes were then transferred into the OPS in 1–2 µl medium and transferred immediately to liquid nitrogen.

Oocytes were warmed by transfer of the vitrified OPS contents into pre-warmed sucrose solutions maintained on a warm stage at 37°C. The oocytes were expelled into 13.69%
and are now in widespread use for embryo cryopreservation for transfer and two implanted and developed to term (5% success rates of around 1% births for conventional freezing or if this represents chromosomal damage as observed for some rapid freezing methods (Shaw et al., 1996).

Mature oocytes and early cleavage stage embryos of many species are sensitive to cryopreservation (Leibo et al., 1996). Vitrification of the extremely sensitive early cleavage stage bovine embryo in a 1–2 µl mixture of ethylene glycol (16.5%), dimethyl sulphoxide (16.5%) and sucrose (0.5 mol/l), in narrow bore plastic straws (OPS) has been shown to be very successful (Vajta et al., 1998). Furthermore, in the same report it was shown that mature unfertilized bovine oocytes could be successfully vitrified in 20% ethylene glycol + 20% dimethyl sulphoxide and 0.5 mol/l sucrose using the same OPS rapid cooling system, if cumulus cells were removed during maturation in vitro. When warmed after vitrification, 25% of oocytes fertilized and developed to blastocysts, compared with 48% of non-vitrified (fresh) oocytes. The blastocysts were reovitrified and they retained the capacity to develop to normal calves at term (Vajta et al., 1998). The mixture of ethylene glycol (40%) and 0.6 mol/l sucrose is a stable vitrification solution (Kuleshova et al., 1999) with relatively low toxicity to embryos. Survival of 65% of the mature human oocytes after vitrification is higher than most reports for cryosurvival of human oocytes (Porcu et al., 1998a). Survival rates after freezing of large numbers of human oocytes by conventional slow cooling or equilibrium cooling methods was 56%, with 63% fertilization after ICSI and a cleavage rate of 90%. Six implanted embryos developed to term from the 709 thawed embryos (Porcu et al., 1998b). Very similar data were reported (Tucker et al., 1998) using the same freezing methods for cryopreservation of human oocytes in 1,2-propanediol. Numbers for fertilization (4/6 oocytes fertilized after ICSI with ejaculated spermatozoa), development to 8-cell stage (3/5 pronuclear oocytes) and development to term (1/3 transferred embryos) in the present study, are encouraging and worth following up because they are in the upper range for rates of survival and development to term of other published cryopreservation methods. The birth rate of 1/17 (6%) vitrified oocytes may indicate that substantial improvements can be achieved to the present developmental success rates of around 1% births for conventional freezing–thawing of human oocytes (Porcu et al., 1998b; Tucker et al., 1998).

Vitrification has been used to cryopreserve human 4- and 8-cell embryos (Mukaida et al., 1998): 40% ethylene glycol together with 18% Ficoll and 0.3 mol/l sucrose were used as a low toxicity solution with stable vitrification properties. Of 52 vitrified embryos, 42 (81%) were considered suitable for transfer and two implanted and developed to term (5% implantation/birth rate). It remains to be determined whether the relatively low implantation rate can be improved for human embryo vitrification or if this represents chromosomal damage as observed for some rapid freezing methods (Shaw et al.,
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References


Kuleshova, L.L., MacFarlane, D.R., Trounson, A.O. et al. (1999) Sugars exert a major influence on the vitrification properties of ethylene glycol-based solutions and have low toxicity to embryos and oocytes. Cryobiology, 38, 119–130.


1991b). However, it should be noted that this chromosomal cryodamage was caused by inadequate concentrations of permeating cryoprotectant when rapid cooling and this would not be anticipated where cryoprotectant concentrations are very high for stable vitrification.

PGD was used to assess aneuploidy for X, Y, 13, 14, 15, 16, 18, 21 and 22 (Magli et al., 1998) in the one donated embryo. This embryo was euploid for these chromosomes. The application of PGD to embryos derived from cryopreserved oocytes could be an important quality assurance procedure because of the reported low implantation rate. This may enable an improved selection of euploid embryos and could raise implantation rates by discarding those diagnosed as aneuploid. This might be important for donation of cryopreserved oocytes to provide the recipient with some assurance of a reasonable implantation rate. It is very important to derive reliable data on the potential increase in embryonic aneuploidy resulting from oocyte freezing and vitrification.

This case report identifies the potential use of the vitrification of oocytes for IVF patients, for oocyte donation and the storage of oocytes for patients who are at risk of sterility because of radio- and/or chemotherapy, and those wishing to delay conception for other reasons. The combination of vitrification in a low toxicity solution, rapid cooling in 1–2 µm volumes in OPS, ICSI for fertilization and PGD to avoid aneuploidy for some chromosomes, enabled the birth of a healthy baby girl for a 47 year old recipient after oocyte donation.