Vitrification of embryos and oocytes with 5.5 mol/l ethylene glycol and 1.0 mol/l sucrose

Dear Sir,

Chen et al. (2000a) have erroneously cited the group of Martino et al. (1996) as the original formulators of a vitrification solution consisting of 5.5 mol/l ethylene glycol and 1.0 mol/l sucrose (Martino et al., 1996; Chen et al., 2000a). This vitrification solution was in fact designed by Ali and Shelton (Ali, 1992; Ali and Shelton, 1993a,b,c).

Vitrification can only be achieved by very high, often embryotoxic concentrations of cryoprotectant solutions. Consequently, it was imperative to formulate a non-toxic and efficient vitrification solution for cryopreservation by
vitrification. Ali and Shelton (1993a) undertook a systematic and extensive investigation involving over 6088 permutations and combinations of cryoprotectant solutions in an effort to identify the ideal and least toxic vitrification solution. This study led to the identification of an ethylene glycol-based solution consisting of 5.5 mol/l ethylene glycol and 1.0 mol/l sucrose (called VS14) (Ali and Shelton, 1993a) which was the least toxic and most versatile of the 6000 solutions investigated.

This vitrification solution was used to cryopreserve all pre-implantation stages of in-vivo generated mouse (Ali and Shelton, 1993b) and day 6 sheep embryos (Ali and Shelton, 1993c) with insignificant loss of viability in vitro or in vivo. Subsequent investigations suggested it to be useful for the cryopreservation of in-vitro generated day 2 embryos and established cell lines in the human (Ali et al., 1995; Ali, 1996a,b). Following these reports, a number of publications appeared on cryopreservation by vitrification of mammalian oocytes and embryos—including human—with the birth of live young, using a vitrification solution consisting of 5.5 mol/l ethylene glycol and 1.0 mol/l sucrose (Papis et al., 1995; Martino et al., 1996; Hong et al., 1999; Chen et al., 2000a,b; Choi et al., 2000; Chung et al., 2000; Yoon et al., 2000). With a notable exception (Papis et al., 1995), all other workers appear to be incorrect in their citations as to the originality of the said vitrification solution.

Dear Sir,

In the article of ‘Open pulled straws (OPS) for vitrification of mature mouse oocytes preserve patterns of meiotic spindles and chromosomes better than conventional straws’ (Chen et al., 2000b), we used the vitrification solution of 5.5 mol/l ethylene glycol and 1.0 mol/l sucrose (EG5.5) and cited the appropriate reference (Martino et al., 1996). The recently developed OPS (Vajta et al., 1999) achieving more rapid cooling and warming rates may alleviate the chilling injury of meiotic spindles of oocytes. In addition, OPS expedite the procedures for loading and releasing oocytes that can reduce the exposure of a highly concentrated solution. In our previous study, we employed EG5.5 for vitrification of mature human oocytes using conventional straws, and achieved a high morphological survival rate, fertilization, and early cleavage of vitrified oocytes (Chen et al., 2000a).

Martino et al. (1996) vitrified bovine oocytes using EG5.5 with a support of the microscope copper grid (Martino et al., 1996). With rapid passage of the dangerous temperature zone, the chill-sensitive bovine oocytes reached better growth potential to blastocysts, compared with those using a conventional straw. However, they did not point out the originality of EG5.5. Hong et al. (1999) attained live births from vitrified human oocytes with reference to the above method (Martino et al., 1996; Hong et al., 1999).

Recently, we received the information that the original formulation of EG5.5—called VS14—was devised by Ali and Shelton (1993). Rall and Fahy (1985) first successfully vitrified mouse embryos with the medium consisting of dimethylsulphoxide (DMSO), acetamide and propylene glycol and polyethylene glycol, which required a low temperature of 4°C during the equilibration (Rall and Fahy, 1985). Subsequent investigators made a significant improvement in adjustment of cryoprotectants for reducing toxicity that permitted the equilibration steps performed at the room temperature or 35°C (Kasai et al., 1990; Ali and Shelton, 1993; Ishimori et al., 1993; Vajta et al., 1998; Park et al., 2000; Yokota et al., 2000).

Ethylene glycol, with the characteristics of low toxicity and rapid permeation of the cell, became an important component of vitrification solutions. Some authors used sucrose as a non-permeating agent to facilitate dehydration and vitrification that further reduced the toxicity of ethylene glycol by decreasing its concentration (Ali and Shelton et al., 1993). Some authors mixed other permeating agents, such as DMSO, to reduce the concentration of single cryoprotectant (Ishimori et al., 1993; Vajta et al., 1998; Yokota et al., 2000). The other investigators added a macromolecule, such as Ficoll, in a vitrification solution that was thought to stabilize the glass formation and form a protective coating around embryos (Kasai et al., 1990; Yoon, T.K., Chung, H.M., Lim, J.M. et al. (2000) Pregnancy and delivery of healthy infants developed from vitrified oocytes in a stimulated in vitro fertilization-embryo transfer program. Fertil. Steril., 74, 180–181.

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References