Human ovarian tissue: vitrification versus conventional freezing

Sirs,

In the challenging paper by Wang et al. (2008), the authors have reported about an effective method of vitrification of human ovarian tissue with direct contact of cells in liquid nitrogen, named needle immersed vitrification.

Childbirth after cryopreservation of ovarian tissue is now a reality (Donnez et al., 2004; Demeestere et al., 2007; Meirow et al., 2007; Andersen et al., 2008) and to study the new modifications of cryopreservation protocols is very interesting.

In fact, vitrification is technologically promising, it is simpler and one cryocycle is less time-consuming and cheaper than the conventional freezing method. However, the central goal of the cryotechnology is the preservation of intact follicles, and not the guarantee of simplicity and availability of technology for operator to the detriment of the post-warming quality of follicles.

However, results of the above investigations (Wang et al., 2008, Fig. 2) have shown that proposed vitrification protocol cannot guarantee the storage of viable follicles after warming in contrast to conventional freezing. Analysis of histological preparation evidence that post-warming follicles are far from normality (Paynter et al., 1999): in both ‘post-warming’ follicles presented in Fig. 2B the vacuolization of cytoplasm, especially in the top follicle as well as the chromatin condensation in both follicles, can be noted; the right follicle in Fig. 2C, which is also denoted by authors as normal, has partly damaged cytoplasm.

In our opinion, in the evaluation of normality of cryopreservation protocol it would be better (i) to demonstrate a bigger sector of tissue (see Isachenko et al., 2008a) and (ii) to evaluate the quality of follicles after long-term culture (see Fig. 1 and Isachenko et al., 2007, 2008a).

Besides, in our opinion, the above-mentioned method of vitrification cannot be recommended for use in the medical practice because these protocols presuppose a direct contact with liquid nitrogen, which is a potential source of microbial contamination.

In fact, any technology in reproductive biology and especially in a medical approach must ensure and guarantee the full protection of biological objects from micro-organisms. Liquid nitrogen, which is used for the storage of frozen material, can be a source of contamination by these micro-organisms (Tedder et al., 1995; Bielanski et al., 2003). Filtration or ultra-violet treatment of liquid nitrogen cannot guarantee the absence of contamination of biological material by viruses. Different types of viruses, which are simple and very cryostable structures, may increase their virulence after a direct plunging and storage in liquid nitrogen, such as hepatitis virus, papova virus, vesicular stomatitis virus and herpes virus.

The above vitrification methodology is based on the direct cooling of cells in liquid nitrogen. In contrast, conventional freezing completely avoids the direct contact between the liquid nitrogen and the tissue.

References


Figure 1 Histological micrographs of follicles from ovarian pieces after conventional freezing and culture by Isachenko et al. (2006, 2007, 2008a,b). Bar = 10 μm.

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doi:10.1093/humrep/dep094
Advanced Access publication on April 22, 2009

Reply: Human ovarian tissue: vitrification versus conventional freezing

Sir,
We would like to thank Prof. Isachenko et al. for their interest in our study and the issues that they raised in their letter.

Conventional slow freezing method has been applied in human ovarian tissue cryopreservation for more than 10 years. Childbirth after cryopreservation of ovarian tissue through slow freezing was reported since 2004 (Donnez et al., 2004). However human ovarian tissue cryopreservation protocols are still experimental, further modifications are required. Slow freezing protocol needs programmed freezer, which is expensive and time-consuming. So we are afraid that it could not be applied widely in an overpopulated developing country such as China. On the other hand, the vitrification method is convenient and time saving, we consider it might be a more practical technology and has the potential advantages in the medical approach.

As Prof. Isachenko points out the central goal of cryo-technology is the preservation of intact follicles. The integrity of the follicle was evaluated with histological analysis by haemotoxylin–eosin (HE) staining and ultrastructural observation in our study. We noticed that the vacuolization of cytoplasm in oocyte appeared in both the fresh and the frozen-thawed groups analysed by HE stain. It seems that this phenomenon happened in the fixation and the stain procedures, as we consulted with the pathologist. So the authors performed the ultrastructural evaluation. Our results proved that the oocyte was well preserved by needle immersed vitrification (NIV) method. Only HE stain evaluation might be insufficient.

Besides, the ovary has heterogeneous cellular components. It is essential to evaluate the effect of freezing/thawing protocols by estimating both follicle and its surrounding stromal cells. In a comparison between conventional cryopreservation and vitrification techniques in tissue-engineered blood vessels, the vitrification method showed a much better result in preserving multicellular tissues (Dahl et al., 2006). So, we think the vitrification techniques might have potential advantages in preserving ovarian tissues. This was also supported by our preliminary experimental results.

The developmental potential of follicles after cryopreservation is important. To evaluate the cryopreservation protocol, the heterotopic allografting procedure in mouse model was used in our study. We agree with Prof. Isachenko’s opinion that for evaluation of cryopreservation protocol, it would be better to demonstrate a bigger sector of tissue and to evaluate the quality of follicles after long-term culture. We did the ovarian tissue culture procedure after vitrification in groups with different concentrations of cryoprotectant. A manuscript including this part of data has been submitted for publication.

Just as Prof. Isachenko said at the beginning of his letter, we would like to say that the vitrification is less time-consuming and cheaper than the conventional freezing method. Moreover, NIV method could minimize the volume of cryoprotectant. By exposing tissue directly to liquid nitrogen, the cooling rate could be increased and the toxicity could be reduced with lower concentration of cryoprotectant. Another advantage of this method is to ensure that all tissues thread through needles exposing to cryoprotectants synchronously, so that vitrification effect stabilized. The above advantages of NIV method have initially appeared in our published and present research works.

Exposure of tissue/cells directly to liquid nitrogen may raise biosafety concerns and may not be acceptable in some countries. Some vitrification protocols such as the Cryotop method had resulted in healthy babies after cryopreservation of human blastocysts (Steinhil et al., 2005) and resulted in high survival rate and normal births after vitrification of human oocytes (Antinori et al., 2007). This method also carries potential safety concerns, and the authors raise a possible solution to separate cooling and storage phases of vitrification (Kuwayama, 2007). For a future clinical application of the NIV method, the cooling aspect should also be performed in a small volume of sterile or sterilized liquid nitrogen. After vitrification, the device could be sealed into a sterile pre-cooled vial for hermetrical isolation at storage. We agree with Prof. Isachenko that filtration or ultra-violet treatment of liquid nitrogen cannot guarantee the absence of contamination of biological material by viruses. But we believe that there will be a solution in the near future by improving the sterilization techniques. On the other hand, a short-term culture and the detection of microbes before transplantation might do us a favour now.

Finally, we should say it is our honor to discuss the above issues with Prof. Isachenko and colleagues.

References