Reproductive BioMedicine Online (2015) **30**, 325–333

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REVIEW

Open versus closed systems for vitrification of human oocytes and embryos

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Abstract Vitrification is now the dominant approach for cryopreservation of human oocytes and embryos; however, serious disagreement persists, particularly about biosafety issues. Techniques are categorized as either 'open' or 'closed' according to occurrence of direct contact between the medium and liquid nitrogen during cryopreservation. Advocates of closed systems emphasize the potential danger of disease transmission mediated through liquid nitrogen, and praise the safety of their approach; those who use the open systems refer to the lack of evidence of disease transmission and regard their systems as more consistent and efficient. The purpose of this review is to clarify whether open and closed systems are really open and closed; if closed systems are safe and free of any danger of contamination; if closed systems are equally efficient as open ones for cryopreservation of human embryos and oocytes by considering overall outcome; and finally, if ethical and legal concerns are sound when risks and benefits are considered in a broader sense. On the basis of these answers, implementation of rational measures to lower the theoretical danger of disease transmission are proposed while maintaining the achievements in cryopreservation that have contributed substantially to the advancement in assisted reproduction techniques during the past decade.

KEYWORDS: biosafety, closed, cryopreservation, disease transmission, open, vitrification

http://dx.doi.org/10.1016/j.rbmo.2014.12.012

Introduction

The application of vitrification considerably improved the in-vitro and in-vivo development of cryopreserved blastocysts and oocytes, and opened new perspectives for extended embryo culture, single blastocyst transfer, blastocyst biopsy, and alternative ways for fertility preservation or oocyte donation, respectively (Arav and Natan, 2013; Chian et al., 2014; Schoolcraft and Katz-Jaffe, 2013; Vajta, 2013).

Currently used vitrification techniques, however, differ from each other in many technical details, including solutions, equilibration and dilution parameters, carrier tools, cooling, storage and warming methods (Vajta and Nagy, 2006). The wide variety of methods makes selection of the best technique difficult, and causes serious problems when cryopreserved samples are transferred between laboratories. One of the most fundamental differences classifies vitrification techniques into two methods: 'open' and 'closed'. Although closed methods are intended to keep the samples physically separated from liquid nitrogen during the entire cooling, storage and warming procedure, open systems allow direct contact between the sample containing medium and liquid nitrogen (Bielanski and Vajta, 2009).

As liquid nitrogen may contain infective agents, direct contact could theoretically mean a possibility for infection and disease transfer. No such disease transfer has yet been reported, although an estimated 600,000 to 1,000,000 vitrified embryos or embryos derived from vitrified oocytes by using open systems have been transferred. At present, most embryos and oocytes are vitrified with open systems worldwide, indicating a higher overall efficiency and consistency, although only a few comparisons between the two approaches have been published.

During the past few years, the open versus closed debate has become the subject of emotionally charged confrontations in many forums, which have included various ethical, legal and financial issues. We have been active contributors to these debates, have commercial interests (please see declaration), and have published pioneering results in the field; therefore, our opinion may be slightly biased and subject to debate.

The main purpose of this review is not to stimulate debate, but to focus attention on aspects that have not been considered so far, facts and arguments that may considerably help to abandon thinking in rigid categories, and promote the formation of a consensus in this very important issue.

To achieve this goal, we have attempted to answer the following questions: (i) what is the exact definition of an open versus closed system? Is the border evident and sharp? Are devices advertised as 'closed' always closed and always safe (i.e. free of danger of contamination from nitrogen or other sources)? (ii) what is the theoretical and practical risk of disease transmission via liquid or vapour phase nitrogen mediated infection? (iii) are closed systems as efficient as open ones for both human blastocyst and oocyte cryopreservation? (iv) what ethical and legal issues should be considered in selecting the appropriate procedure? and (v) what measures can be implemented to minimize sample infection and eliminate the possibility of cross-contamination to decrease or disclose even the theoretical danger of liquid or vapour phase nitrogen mediated disease transfer?

Open versus closed systems: categories and borders

For most embryologists, the two categories are easily distinguishable. Open systems allow and closed systems eliminate direct contact between the sample-containing medium and liquid nitrogen. Consequently, from a disease transmission point of view, open systems are unsafe, and closed systems are safe.

The situation, however, is much more complicated.

The principle of vitrification in cryobiology is to eliminate totally ice formation in the medium that contains the sample, in all phases (cooling, storage and warming) of the procedure (Rall and Fahy, 1985). It can be achieved either by increased cooling and warming rates, or increasing concentration of cryoprotectants; in practical situations, both approaches are applied. The higher the cryoprotectant concentration, the lower the cooling rate required and *vice versa*. As highly concentrated cryoprotectants may cause toxic and osmotic injury, the preferred strategy is to use the highest possible cooling and warming rates, then to apply the lowest concentration of cryoprotectants that ensures safe ice-free solidification under these circumstances (Fuller and Paynter, 2004; Kasai and Mukaida, 2004; Stachecki and Cohen, 2004; Vajta and Nagy, 2006). High cooling and warming rates may also help to avoid chilling injury (Ghetler et al., 2005).

The easiest way to achieve high cooling and warming rates is to use the smallest solution volume and the highest temperature conductivity between the sample-containing medium and the cooling or warming agent, preferably liquid nitrogen for the former purpose (Arav, 1992).

Decreasing the thickness of the wall of the sample-holding container, for example straw, may be helpful. Obviously, a total elimination of the thermo-insulating layer is the best solution. However, the seemingly easiest approach - small droplets freely plunged into the liquid nitrogen (Landa and Tepla, 1990) - is suboptimal. To form a drop, an excessive amount (>3 μl) of solution is required, and the nitrogen vapour coat that surrounds the warm medium will keep the drop for a relatively long period (8–10 s) over the surface of liquid nitrogen, decreasing considerably the cooling rate. Accordingly, carrier tools were introduced to hold small amounts, and ensure rapid submersion and fast elimination of the vapour coat (Martino et al., 1996; Steponkus et al., 1990). The small (>1, > 0.5 μl) amount of solution also helps to minimize the danger of heterogenous ice formation (Rall et al., 1987).

Most carriers are based on homemade, simple tools, later modified for industrial production; however, these modifications did not always increase practical value and safety. At least 30 different carrier tools have been published, and at least 15 versions are commercially available. Most of them are slightly modified versions of the initially introduced carrier tools, such as the Open Pulled Straw (OPS) (Vajta et al., 1998a), the Cryoloop (Lane and Gardner, 2001; Lane et al., 1999), and the Cryotop (Hamawaki et al., 1999). All these systems are open in the original form. Most claimed 'closed' systems are the results of the modifications of these open systems.

A thorough structural and functional investigation of the existing vitrification systems reveals various levels of openness, consequently various levels of biosafety (differences that are commonly disregarded in the laboratory practice). 'Safety' or 'biosafety' in the context of this discussion refers to a